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ON THE CHEMICAL BONDS BETWEEN ANIMAL CELLS. A MECHANISM FOR TYPE-SPECIFIC ASSOCIATION

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INTRODUCTION

In recent years it has been shown (Moscona and Moscona, 1952; Moscona, 1952, 1956, 1957; Weiss and James, 1955; Townes and Holtfreter, 1955) that when vertebrate embryonic cells of diverse types are intermixed, they have the capacity to segregate themselves from cells of other types and reestablish the discrete tissues which they originally represented. Such observations have confirmed and extended previous findings (e.g. Holtfreter, 1939; Weiss, 1947) concerning tissue affinities.

We are confronted with the question of how cells are able to discriminate among their fellows. The selective mechanism must reside in the cell surfaces, for it is the surface which either forms or fails to form connections with the surfaces of other cells which are encountered. But, before we can discover wherein the selectivity of the cell surface lies, we must first know the general mechanism by which cells are held together. The specificities may then be found in the subtle manipulations of this mechanism by the cells.

Weiss (1941, 1947) has provided us with an ingenious series of schemes by which many of the phenomena involved in cell associations can be adequately accounted for. Tyler (1940, 1942, 1946a, 1946b) has expressed similar views. In brief, Weiss postulates that cell surfaces contain sterically specific configurations which combine with complementary configurations on the surfaces of other cells in a manner analogous to the combination of antigen with antibody. Attachments between two unlike cells are envisioned as involving the "keys" on the surface of one cell and the "locks" on the surface of the other ("complementary affinity"). Attachments between two like cells ("homonomic affinity") are conceived of in two alternative ways. Each surface may be provided with both "locks" and "keys," interaction within the same surface being prevented by the orientation of the reactive groups; or the surfaces may contain only "locks," the "keys" being represented in a substance extruded between the two surfaces

and acting as a specific cement. Differences in the strength of binding between a given cell and cells of several different types are viewed as being due to differences in the relative abundance or in the spatial configuration of the complementary binding groups on their respective surfaces.

Attempts to subject the above views to experimental test have been few. Spiegel (1954a, 1955) has reported studies of great interest, in which he finds it possible to inhibit reversibly the reaggregation of sponge cells with the corresponding immune sera. He has also carried out less extensive studies (Spiegel, 1954b) with embryonic amphibian cells. However, the inhibition of reaggregation by antisera directed against the cells concerned is not a demonstration that the process of aggregation itself is analogous with the combination of antigen with antibody; nor is it a demonstration that the antiserum has reacted with the surface groups specifically responsible for aggregation. The possibility remains, and it has been recognized by Spiegel (1954a, p. 145; 1955, p. 1077), that the antiserum reacts with groups *neighboring* those responsible for aggregation and by steric hindrance prevents the latter from functioning.

In view of the present condition of our knowledge concerning the attachments and the affinities between cells, a reexamination of the subject seems justified. We should gain insight into what holds cells together in a tissue fabric by an examination of the methods which have been successful in dissociating that fabric and of the conditions under which the cells will again resume their associations. Information from other sources pertaining to the structure and properties of cell surfaces will be valuable in this connection. The picture of cell-cell attachments which emerges from this examination may then provide a clue to the nature of those modifications of the basic attachment mechanism which could afford the selectivity which we know exists.

The questions which we shall pursue, then, are these: "What may be the forces which unite cells into tissues," and "By what mechanism may cells exert preferences in their associations with other cells?"

METHODS OF TISSUE DISSOCIATION

1. *Mechanical Disruption of Tissues.*

Beginning with the experiments of H. V. Wilson (1907) on sponge dissociation and reaggregation, many workers have disrupted tissues mechanically into suspensions containing various proportions of tissue fragments, small groups of cells, isolated cells and debris. Whereas many questions concerning the reaggregation of dissociated cells, particularly in the sponges, have been answered by such investigations, this method of causing dissociation provides us with no information concerning the nature of the original cell-cell attachments.

2. *Removal of Alkaline Earth Cations.*

Ringer (1890) and Herbst (1900) were the first to demonstrate dissociation of tissues in Ca-free media. Since that time many others have observed

the same phenomenon in a variety of tissues. Gray (1926) obtained dissociation of Mytilus (mollusk) gill epithelium in the absence of Mg from the medium. Ca seemed to play a lesser role in this instance. In the case of amphibian embryos, dissociation occurs in Ca-free solution, but other means of dissociation are more efficient (Holtfreter, 1943, p. 297; 1948). Removal of plurivalent cations by complexing agents greatly facilitates separation of the cells (Feldman, 1955; King and Briggs, 1955). The chick embryo may also be dissociated by chelating agents (Zwilling, 1954), as may the mouse embryo (Auerbach and Grobstein, 1958) and the blastomeres of early cleavage stages of the rabbit embryo (Brochart, 1954). Chelating agents also loosen the connections between cells of adult vertebrates (Anderson, 1953), which can be loosened significantly in Ca- and Mg-free solution in the absence of complexing agents (Zeidman, 1947). In the case of human buccal epithelium, both Ca and Mg seem to contribute to intercellular binding (Zeidman, 1947).

In all systems studied to date and familiar to the writer, the presence of Ca (or Mg) has proved essential for reaggregation. Sponge cells may represent an exception to this statement, since Spiegel (1954 a, 1955) has found that absence of Ca and Mg, even in the presence of complexing agents, prevents only the cell movements which are normally responsible for bringing dissociated cells into mutual contact. According to Spiegel, if the cells of such preparations are brought into contact mechanically, they adhere to one another. This case certainly deserves further study, since the cells both of amphibian and of chick embryos are mutually nonadhesive in Ca- and Mg-free culture solutions, the former showing amoeboid activity under these conditions (author, unpublished). Despite the apparently unique behavior of sponge cells, the conclusion seems generally warranted that Ca and/or Mg are intimately involved in the links which unite cells into tissues. Any satisfactory theory of the mechanism of cell-cell binding must account for the action of alkaline earth ions, and in particular of Ca.

3. *Alkaline Solutions.*

In his studies on Mytilus gill epithelium, Gray (1926) found that alkaline solutions promote dissociation of the cells. Holtfreter (1943, 1944 a, 1944 b) has shown that the cells of amphibian embryos will dissociate if the pH of the ambient medium is raised to about 10. Mookerjee (1953) has duplicated these results with the chick embryo, a pH of 9.0–9.2 sufficing in this case. Essner, Sato and Belkin (1954) obtained dissociation of the cells of a rat hepatoma at a pH of around 9 or above.

Dissociation by alkaline solutions is not readily conceived of as being a special case of dissociation by removal of Ca, since insoluble $Ca(OH)_2$ is not precipitated until appreciably higher pH levels are reached. $Mg(OH)_2$ precipitates at more acidic levels than $Ca(OH)_2$, but is still quite soluble at pH 9, where some types of cells dissociate. Further discussion of dissociation by alkaline solutions will be reserved for a later section.

4. *Trypsin.*

The use of trypsin for the liberation of free tissue culture cells by digestion of the plasma clot was introduced over forty years ago by Rous and Jones (1916). More recently Moscona (1952) has developed a technique for tissue dissociation which has enjoyed widespread use. This method, designed for use with the chick embryo, is generally thought of as a "tryptic dissociation." It involves incubation in Ca- and Mg-free Tyrode's solution at a pH of around 8.5 in the presence of crude "trypsin," final dissociation being achieved mechanically. There are several points concerning this method which deserve mention, but they too will be discussed later.

THE CELL SURFACE

1. *Electrical Charge.*

Electrophoretic measurements on a variety of animal cells have shown that cell surfaces are, generally speaking, negatively charged at physiological pH. The eggs, larvae and adults of a wide variety of parasites bear negative charges (Senekjie and Scott, 1942), as do the eggs of many echinoderms (Dan, 1933, 1934, 1947a, 1947b), an annelid (Nereis) and a mollusk (Cumingia) (Dan, 1934). The unfertilized egg of Cerebratulus, a rhynchocoel, bears little or no charge (Dan, 1934), whereas frog eggs bear a positive charge (Mazia, 1933). The frog egg, however, is covered with a surface coat (Holtfreter, 1943) distinct from the cell membrane, so that its electrical properties do not provide information pertinent to the cell membrane itself.

The spermatozoa of echinoderms (Gray, 1915; Mudd, Mudd and Keltch, 1929) are negatively charged, but the situation in the mammals is confused. Shreder (cited in Gordon, 1957) has found that at neutral pH a suspension of rabbit spermatozoa will split into two parts, one moving toward the anode and the other toward the cathode. These two fractions were claimed to represent predominantly X- and Y-bearing sperm, respectively. Gordon (1957) has recently supported this claim. However, Machowka and Schegaloff (1935) offer detailed evidence that dog spermatozoa are negatively charged but swim toward the cathode, so that inactive cells accumulate at the anode and active cells at the cathode. Upon heat-killing, all of the spermatozoa accumulate at the anode. The same authors state that human spermatozoa are negatively charged but also swim toward the anode, so that in any case they migrate anodally. Mammalian red blood cells also bear negative charges at physiological pH (Coulter, 1920; Abramson, 1934; Byler and Rozendaal, 1938; Furchgott and Ponder, 1941), the isoelectric point of the human erythrocyte being at around pH 1.7 (Furchgott and Ponder, 1941).

To the writer's knowledge the only published information on the surface charges of vertebrate tissue cells comes from the work of Ambrose, James and Lowick (1956), who find that hamster kidney tumor cells dissociated by the method of Anderson (1953) are more highly charged than the corresponding normal cells, and that the same is true of normal and cancerous rat

liver cells. From the text one can conclude that these charges are negative, although this is not explicitly stated by Ambrose *et al.* We have made an electrophoretic study of 5½ day chick embryo liver cells and found (author, unpublished) that they are negatively charged even at pH 4 in dilute veronal-acetate buffer without added electrolytes.

The observations above seem to justify the statement made at the beginning of this section that animal cell surfaces are in general negatively charged. Further, the low isoelectric points indicate the presence of strongly acidic groups at the surfaces. What are the chemical groups to which this charge is attributable, and what bearing might they have upon the question of the nature of chemical bonds between animal cells?

2. *Physicochemical Structure.*

A detailed review of the physics and physical chemistry of cell membranes lies beyond the scope of this article. The reader may find a discussion of the subject in a recent paper by Mitchison (1952; but see Geren and Schmitt, 1954, p. 867). It will serve our purpose merely to point out the major components of cell membranes and something about their orientation.

The classical object for the study of the structure of the cell membrane has been the red cell ghost, which has until recently been the only such material known to be readily procurable in large quantities. The studies of Geren, however, have shown (Geren, 1954, 1956) that the myelin sheath of nerve is the spirally wound Schwann cell membrane. Physical studies of myelin (Schmitt, Bear and Clark, 1935; Schmitt, Bear and Palmer, 1941; Schmidt, 1937; Fernandez-Moran and Finean, 1957), therefore, provide information about the structure of the Schwann cell membrane. Chemical analysis of red cell ghosts (Parpart and Dziemian, 1940) shows the presence of large amounts of phospholipid, cholesterol and protein and a very small amount of carbohydrate. The general conclusions from polarized light studies of ghosts (Schmitt, Bear and Ponder, 1936) coincide with those based on myelin (Schmidt, 1937), namely that the structure is one in which lipid forms a radially oriented phase while protein micelles are tangentially arranged. The topographic arrangement of protein and lipid in the membrane remains conjectural. The wetting properties of the red cell surface (Mudd and Mudd, 1926) indicate that lipid is exposed. Its reaction to lipase (Ballantine and Parpart, 1940) may be similarly interpreted. On the other hand, the electrophoretic mobility of intact human red cells, except at rather low pH, is intermediate between the mobilities of stromal protein and extracted lipids (Furchgott and Ponder, 1941). This may be taken to mean that protein is exposed as well as lipid.

We may conclude from the above that the external membrane of an animal cell is a highly ordered structure, the negative charge of which is largely referable to ionized groups of phospholipid and protein. These groups will be, for the most part, the phosphoric acid group of "cephalin" and the carboxyl groups of proteins.

THE ROLE OF CALCIUM IN INTERCELLULAR BINDING

From what has been said above there emerges a portrait of animal cells in which the surfaces are provided with ionized phosphoric acid and carboxyl groups. Such a negatively charged surface would attract cations, bivalent cations being more effective in neutralizing a negatively charged surface than monovalent cations. But with bivalent cations, such as Ca, there is, in addition, the opportunity for a chemical reaction. Slightly soluble calcium salts may be formed with the surface anions. Such a situation has been described in solutions of ovalbumin on the alkaline side of its isoelectric point (Danielli, 1944), and it has been proposed (Höber, 1945, pp. 305–306) that this process may stabilize the intercellular matrix which is imagined as cementing cells to one another. Schmitt (1941) has suggested that Ca may act to desolvate the cell surfaces, removing the water of hydration and thus allowing the intimate contact between two surfaces which would be necessary for adhesion to occur (presumably as the result of unrelated reactions). It would appear, however, that a more specific involvement of bivalent cations must be sought, since Dan (1947) has shown that when the surfaces of sea urchin eggs are brought to the same electrical state with different cations they show characteristic differences in adhesiveness. The cations have specific effects apart from electrostatic ones.

Ca (or Mg, or both), in addition to desolvating the cell surfaces, associates with the surface anions. If certain of these anions are sufficiently separated from one another, the formation of slightly soluable salts involving two anions and one Ca would not be possible. When two cells in such a state collide, however, the formation of slightly soluble Ca salts *between* the two surfaces becomes possible, the two valences of Ca being satisfied by an acidic group on the surface of each of the two cells. I wish to propose that this is the mechanism by which the cells are held together, the anions involved being either carboxylate or the substituted phosphoric acid group of "cephalin."

This proposal raises questions to which answers may be attempted. First of all, what becomes of the intercellular matrix which has been imagined to cement cells together? An examination of the literature on this subject has not revealed any clear-cut distinctions between a supposed matrix and the surfaces of the cells themselves. In this connection an observation by Geren and Schmitt (1954) is of the utmost interest. These authors observed with the electron microscope squid giant nerve fibers which had been treated with artificial sea water devoid of Ca or Mg ions, or which had been fixed in a solution containing sufficient Versene (EDTA; ethylene diamine tetraacetic acid) to chelate the alkaline earth ions present. They observed that the apposed, dense-edged, Schwann cell membranes in such a preparation were spread apart from each other. In the case of the giant fibers of the squid, the surface of the Schwann cell is "complexly infolded to form a system of intracytoplasmic double membranes" (Geren,

1956, p. 218). Here one would hardly expect to find "intercellular cement," but removal of alkaline earth ions has the same effect upon these cell membrane pairs as upon the apposed membranes of adjacent tissue cells.

It is appropriate to point out in this connection that electron micrographs of the junctions of tissue cells show approximations quite as close (between the osmiophilic layers) as those in Geren and Schmitt's figures of squid Schwann cell membranes. In the case of the membranes which comprise myelin, there can be little doubt that they are in direct contact with each other. In view both of their behavior toward lack of alkaline earth ions and of the similarity between the degree of their intimacy and that of the membranes of adjacent tissue cells, the conclusion that cells are bonded directly surface-to-surface would seem to be considerably strengthened.

We have undertaken some experiments on newt embryo cells in an attempt to evaluate the proposal which has been made. If Ca functions as the salt of an acid, then it should be possible to prevent reaggregation of cells dissociated with EDTA by suppressing the ionization of the acid.

Portions of the marginal zone of early gastrulae of the Japanese newt *Triturus pyrrhogaster* were dissociated with EDTA, washed in Ca-free Holtfreter's solution, and immersed in an appropriate salt solution (a modified Holtfreter's) at pH values ranging from 4 to 10. Ca and Mg were both present. Reaggregation proceeded normally from pH 6 to pH 10. (In this species alkaline dissociation does not occur until a pH near 11 is reached.) At pH 5.5 reaggregation was reduced; at pH 5.0 it was more severely reduced; and at pH 4.5 it was scarcely evident. At pH 4.0 no reaggregation occurred. After 2½ hours the solutions from pH 4.0 to pH 5.5 were replaced by solutions of the same modified Holtfreter's at pH 7.3 (Tris buffered). After a considerable delay the cells in each case proceeded to reaggregate.

These results support the view that Ca functions as the salt of an acid. In view of the delay in reaggregation after low pH treatment, it would be premature to conclude that the pH dependence curve obtained here represents the dissociation curve of the acid involved, although this is distinctly possible. It should be mentioned that Holtfreter (1948) has reported reversible dissociation of amphibian embryo cells at a pH below 4.2.

If Ca functions as a direct link between acidic groups on adjacent cell surfaces, other bivalent cations should act similarly, although with different degrees of effectiveness. A number of bivalent metal chlorides have been tested in this connection. Each was used as an equimolar replacement of $Ca(Cl)_2$ in unbuffered Holtfreter's solution at pH 7.3. Entire late blastulae and early gastrulae were dissociated as above and then washed in the solution to be tested, which was then replaced with fresh solution. In all solutions the first stages of reaggregation occurred, but toxic effects of some of the ions were soon apparent. The order of effectiveness of these cations in promoting aggregation, as measured by the time required for onset of aggregation, was Ca > Ni > Co > Zn > Mg. Ni and Co were as effective

as Ca in initiating reaggregation, but soon lagged behind, presumably because of their toxicity at this concentration ($9 \times 10^{-4} M$). Zn, on the other hand, seemed to be much less toxic.

These observations are also in accord with the view which has been outlined, although they by no means exclude other interpretations. One might still contend that the bivalent cations induce in the cell surface a unique condition in which nonionic binding sites are properly displayed. While such a theory is tenable, it is more complex than the one which has been proposed.

SELECTIVE CELL ASSOCIATIONS

Weiss' proposed explanation of cell affinities has already been outlined. His theory can satisfactorily account for the selectivity of association which tissue cells display, but takes no account of the role of Ca in intercellular binding. He writes (1950, p. 184), "... the primary adhesive forces are secondarily reinforced and perhaps fully supplanted by intercellular fiber cements. Calcium seems to be an important factor in this process, but can obviously not account for the selectivity of the bonding." We shall now attempt to show how the observed selectivity can be accounted for without the introduction of bonds other than those which have been proposed.

The cell surface is a highly organized structure, as has been pointed out above. A recent observation by Fernandez-Moran and Finean (1957) has suggested a still higher degree of orientation in the tangential direction than previous studies have revealed. In electron micrographs of cross sections of myelin exposed to several different experimental procedures, these authors observed a breaking-up of the electron-dense layers into series of granulations. The granules are fairly uniform in size and "frequently give rise to a periodicity of 60 to 80A along the layers." It is further noted that the strong 60 to 70A vector indicated by x-ray diffraction data is not accounted for by the known radial organization of the lamellae, but might well be supplied by a periodicity such as that observed in the tangential direction. Thus, these observations suggest a regular lattice arrangement of the cell surface.

Let us now assume that the cell surface is indeed a latticework and that the negatively charged groups to which Ca is linked, being at specific points within regularly arranged molecules, are also regularly spaced. They may be envisioned as being at the intersections of a grid projected upon the surface of the cell. Let us further assume that the periodicity of this lattice is peculiar to the specific cell types at specific stages in their differentiation, or at least that a given periodicity simultaneously characterizes no more than a few cell types which are at that developmental stage not normally in contact with one another.

When two similar cells are brought together, the lattices on their surfaces being congruent, the sites of Ca binding on one cell are directly apposable

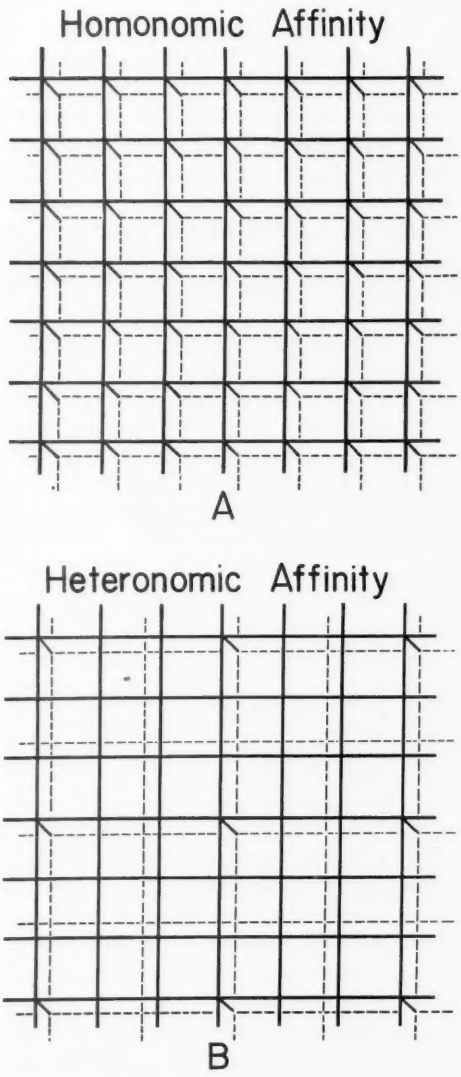

FIGURE 1. (A) Representation of points of binding between surface lattices of two similar cells. (B) Representation of points of binding between portions of two cells whose surface lattices have unit spacing of 2 and 3 arbitrary units, respectively.

to corresponding sites on the other, with the result that a slightly soluble Ca salt is formed between the two surfaces at each intersection of our projected grid (figure 1a). When two dissimilar cells come together, the lattices are *not* congruent. Depending upon the relative dimensions of the two lattices, a greater or fewer number of Ca links can be made. If the periodicities of the two lattices are appreciable factors of a common unit, then a considerable number of Ca links will still be made, and the cells will adhere with corresponding tenacity (figure 1b). Should the periodicities of the two lattices be incommensurable, very few calcium links will be made, and these two cells will have very little tendency to adhere to one another. The three examples which have just been given correspond respectively to "homonomic affinity," "complementary affinity," and "disaffinity" in the terminology adopted by Weiss, and represent only three conditions selected from a continuum. In view of the implications of the term "complementary," "heteronomic" might more precisely apply in the present instance.

It is appropriate here to point out certain similarities between the models provided by Weiss (1941, p. 189; 1947, p. 257) and the one presented above. Homonomic affinity in the present scheme is analogous to Weiss' model C if the ligands are taken to be equivalent and the intermediate units are taken to represent Ca ions. Heteronomic affinity as it is represented here might be compared with Weiss' model D.

A peculiar advantage of the scheme outlined above should be indicated. It is commonly observed (Moscona and Moscona, 1952; Moscona, 1956, 1957; Weiss and James, 1955) that, in addition to the sorting-out of the cells of a heterogeneous aggregate to form islands of discrete tissues, the particular reconstituted tissues are preferentially arranged within the aggregate. For instance, cartilage usually assumes an internal position unpenetrated by masses of other cells, epithelial tissues assume a peripheral location, and connective tissue cells take up intermediate sites.

Such preferential positioning would be expected on the basis of the theory of intercellular binding which has been proposed here. Let us, as an illustration, consider three types of cells, which we may call X, Y and Z, mixed together and allowed to reaggregate. We shall ascribe to the surface lattices of these cells unit dimensions of 2, 3 and 4 arbitrary units, respectively. That is to say, the distance between adjacent sites of potential intercellular Ca salt formation is 2 arbitrary units on the surface of cell type X, etc.

Table 1 shows the number of intercellular bonds per unit area which can be formed between all possible combinations of X, Y and Z. The greatest number of bonds per unit area possible in this assortment is between two cells of type X, in which 1 bond may be formed per area of 4 square units. Consequently, cells of type X will, upon mutual contact, adhere the most tightly and will resist interpenetration by Y or Z cells. Looked at another way, an X cell would displace a Y or Z cell from an already established contact with another X cell. The result would be the formation of one or

more solid islands of X cells surrounded by Y and Z cells. Of these latter types, Z can bond to X more than twice as tightly (more than twice as many bonds per unit area) as can Y. In consequence cells of type Z would displace those of type Y from the surface of a cell or an island of type X. But Z cells can bond to one another 9 times as tightly as to Y cells, so that they would further drive Y cells to the periphery of the aggregate. Nevertheless, Y cells bind to one another very strongly (almost one half as strongly as X to X), and would, therefore, constitute a good epithelium at the periphery of the aggregate. As can be seen, X might represent cartilage, Y, epidermis, and Z, connective tissue. It is clear that the relative positions taken by cells of diverse types in a reaggregate do not necessarily reflect the relative abundance of these Ca-binding sites on their surfaces, although in binary combinations the central component should have the greater abundance of such sites. Investigations into this matter are planned.

TABLE 1

	X ($\frac{1}{2}$)	Y ($\frac{1}{3}$)	Z ($\frac{1}{4}$)
X ($\frac{1}{2}$)	$\frac{1}{4}$	$\frac{1}{36}$	$\frac{1}{16}$
Y ($\frac{1}{3}$)	..	$\frac{1}{9}$	$\frac{1}{144}$
Z ($\frac{1}{4}$)	..	..	$\frac{1}{16}$

Hypothetical representation of areal frequency of bond formation between combinations among cells of three types. Bracketed fractions indicate number of bonding sites per arbitrary unit linear distance along axis of cell surface lattice. Unbracketed fractions indicate number of potential links per unit surface area between combinations of cells.

How well does this view of intercellular binding agree with the facts of dissociation and reaggregation as we know them? Cells held together by this means would dissociate in the absence of Ca (or Mg, or both, as the case may be). They would reaggregate when the appropriate alkaline earth cation(s) again became available. Other bivalent cations would promote aggregation in various degrees, provided that toxicity did not interfere. Cells would either dissociate or fail to aggregate at sufficiently low pH, this effect being reversible if the appropriate pH is not too extreme. In aggregating they would first, in most cases, form random associations, subsequently sorting out into discrete tissues. Finally, the reconstructed tissues would not be randomly arranged in the aggregate, but would occupy defined positions with respect to one another. All of these properties are, in fact, characteristic of the behavior of embryonic cells. There are, however, two general phenomena which the present theory does not predict. These are dissociation at high pH and dissociation by "trypsin." We may now offer possible explanations for both of them.

THE EFFECT OF ALKALINE SOLUTIONS

Early in this article, it was pointed out that dissociation by means of alkaline solutions is apparently not due to the removal of alkaline earth cations by precipitation. It has usually been supposed (Gray, 1926, 1931; Essner *et al.*, 1954) that at elevated pH the "intercellular cement" is dissolved or dispersed. The evidence for the existence of intercellular cement (as distinguished from the intercellular substance of connective tissue) is hardly convincing, but, even if we were willing to grant its presence, we would be hard pressed to explain how reaggregation can so readily occur, upon lowering of the pH, once the "cement" has been removed.

For an explanation of alkaline dissociation, we may turn to observations by Holtfreter (1943, p. 296; 1948) and by the writer. Holtfreter (1948, p. 717) writes that embryonic amphibian cells placed at a pH above 9 "swell considerably through increase of ectoplasmic fluid. A large hyaline cap is formed which rotates rapidly around the surface..."; and again (p. 748), "The general rule that cellular adhesiveness decreases with the swelling of the cell likewise applies to the different regions of the individual cell, for the surface of an expanded hyaline bulge is nonadhesive in contrast to the more contracted region of the cell."

We have made observations on the surface motility of isolated cells of *Triturus pyrrhogaster* gastrulae in Holtfreter's solution brought to different pH values with HCl and NaOH. At pH 4 no movement can be seen. At pH 5 membrane motility can scarcely be detected, and then only by prolonged concentration upon a single cell. From pH 6 up through pH 8 or 9, the slow movement of pseudopodia or "hyaline bulges" is readily observed, but as the pH is raised to 10 and, finally, to 11 the activity of the membrane becomes almost frantic. Pseudopodia appear, rush around the cell and disappear, only to be replaced by new ones.

Here may be found the explanation for dissociation at high pH. In the case of fibroblasts, according to Abercrombie and Heaysman (1954a, 1954b), cell surfaces are somehow stabilized against amoeboid movement as a consequence of their contact with the surfaces of neighboring cells. It may be proposed that the same is true of other normal cells. In fact, Holtfreter (1943, figure 13) has observed just such a phenomenon in amphibian embryonic cells. When the pH of the ambient solution is raised, the tendency toward amoeboid movement is progressively increased until at some point the stabilizing influence of the contiguous cell surfaces is overcome. The cells swell, and processes are put forth. Under such conditions the surface membranes are irregularly deformed, the lattice pattern is distorted, the geometric conditions allowing for attachment are no longer present, and the cells separate.

THE EFFECT OF "TRYPSIN"

The action of trypsin, like that of alkali, has been presumed to be upon the "intercellular cement," which trypsin is supposed to digest. But here

too it is difficult to see how after digestion of the cement which is alleged to hold the cells together they nevertheless, given appropriate conditions, promptly reaggregate. Let us examine the conditions under which "trypsin" is commonly used.

First, it must be said that the "trypsin" employed by Moscona and most of those who have used his technique is a relatively crude pancreatic extract, the enzymatic heterogeneity of which can scarcely be doubted. Such a preparation is more aptly termed "pancreatin." Crystalline trypsin has been employed in a modification of Moscona's procedure (Weiss and James, 1955), but it has been shown (Auerbach and Grobstein, 1958) that the cruder preparation has desirable properties not found in the crystalline enzyme, which is effective, however, in promoting dissociation. Thus, it would be inaccurate to conclude that the efficacy of the crude preparations is entirely attributable to trypsin, or perhaps even to proteases, alone.

In addition, it must be pointed out that Moscona's procedure involves incubation of the tissue for 15 to 20 minutes at pH 8.4–8.6. As has already been mentioned, the dissociation of chick embryo cells is reported to occur within a few minutes at pH 9–9.2 in the absence of extraneous enzymes (Mookerjee, 1953). The cells of a hepatoma (Essner *et al.*, 1954) dissociate under similar conditions. Next, it should be noted that the entire procedure, which occupies approximately three quarters of an hour, is carried out in a medium free of Ca and Mg, a condition in itself conducive to dissociation. Finally, and perhaps critically, the buffer employed is phosphate. Essner *et al.* (1954) have shown that rat hepatoma islands do not dissociate in response to trypsin dissolved in various isotonic salt solutions or in buffer solutions *other than phosphate.* Isotonic phosphate buffer, however, causes a considerable dissociation of these cells *in the absence of trypsin.* The obvious conclusion is that the phosphate is acting to complex Ca (and perhaps Mg) from the tissue.

It seems clear that even in the absence of added digestive enzymes the above procedure would result in a considerable loosening of the tissue fabric. What role may be played by the trypsin and other enzymes present? Auerbach and Grobstein (1958) have found that an enzyme other than trypsin present in pancreatin removes "gum" produced during the dissociation procedure. This "gum" entrains the loosened cells and prevents their clean isolation. Crystalline trypsin, however, was found to be more effective than pancreatin in promoting the initial separation of the cells. Yet trypsin alone seems to be ineffective in causing dissociation (Essner *et al.*, 1954), at least in the case of a rat hepatoma. If trypsin normally acts upon an extracellular protein, it should cause dissociation in salt solutions and in buffers other than phosphate. The fact that it does not, in at least one case, and that the other features of Moscona's procedure cause significant loosening of the cells, suggests that trypsin may only facilitate the separation of already loosened cells by a "de-gumming" process such as that described above, the "gum" being colloidal material which has leaked out

of the cells due to the increased permeability caused by high pH and lack of Ca.

I do not wish to deny the possibility that trypsin may also have a more direct action than the one attributed to it here. I only wish to draw attention to the conditions under which it has appeared to be so effective and to those under which it appears to be ineffective, since a lack of appreciation of those conditions may conceivably mislead us in our conclusions concerning the bonds which unite animal cells into tissues.

SUMMARY

It is proposed that the surface of tissue cells is highly ordered in the tangential direction. This order is reflected in a lattice arrangement of certain ionized acidic groups which bind Ca or Mg. The lattice spacings are peculiar to specific cell types at specific stages in their differentiation, and wide enough to preclude the formation of slightly soluble Ca or Mg salts involving two acidic groups on the same cell surface. It is suggested that the binding of cell to cell is achieved through the formation of slightly soluble Ca or Mg salts between the surfaces of adjacent cells, and that the strength of intercellular binding is consequently dependent upon the relative dimensions of the lattices involved.

On the basis of this model the phenomena of cell dissociation, reaggregation, graded affinities, sorting out of heterogeneous cell populations, and preferential location of reconstituted tissues within aggregates may be explained.

ADDENDUM

After this paper had been prepared, our attention was drawn to the paper of A. S. Curtis (1957, Proc. Roy. Phys. Soc. Edinburgh, 26: 25-48), who reports studies on Ca removal from Xenopus embryos during dissociation with chelating agents, and upon subsequent Ca uptake during reaggregation. He finds, among other things, that both Ca uptake and reaggregation cease, except for the endoderm, in the earliest neurula stages, and that dissociation is no longer provoked by EDTA after neural tube closure. He suggests that a Ca-cement may become operative in late gastrulae and neurulae.

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SALT WATER TOLERANCE OF SEEDS OF GOSSYPIUM SPECIES AS A POSSIBLE FACTOR IN SEED DISPERSAL.*

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An interesting feature of the wild species and wild forms of the cultivated species of Gossypium is the frequency with which they occur as insular or coastal forms (table 1). Inspection of this table reveals another item of interest, namely, that all the forms included are found in the western hemisphere or in Polynesia, that is, they are confined to the American diploid and the allotetraploid New World species. None of the numerous Old World (including Australian) species has any marked preference for island or coastal habitats, though wild species of Gossypium as a whole are restricted to arid conditions.

TABLE 1

INSULAR AND COASTAL WILD FORMS OF GOSSYPIUM. LETTERS IN PARENTHESIS INDICATE CYTOLOGICAL GROUPING: D—AMERICAN DIPLOID, AD—ALLOTETRAPLOID.

Species	Location and habitat
G. armourianum Kearney (D)	San Marcos Island, Gulf of California
G. barknessii Brandg. (D)	Islands and coasts of Gulf of California
G. klotzschianum Anderss. (D)	Galapagos Islands
G. klotzschianum var. *davidsonii* Hutch. (D)	Islands and coasts of Gulf of California
G. aridum Skovsted (D)	Coastal zone of Sinaloa
G. tomentosum Nutt. (AD)	Hawaii—rocky or clay plains near the sea
G. barbadense var. *darwinii* Hutch. (AD)	Galapagos islands
G. hirsutum L. (AD)	
(a) race *morrilli*	Sonora—sand dunes
(b) race *yucatanense*	N. Yucatan coast
(c) race *marie-galante*	Caribbean Islands
(d) race *punctatum*	
(1) "algodon brujo"	Puerto Rico—windswept coastal plain
(2) "taitense"	Polynesian islands
(3) "Florida Wild"	Florida coast, Bahamas

There is good reason to suppose that the coastal and insular forms are not merely tolerant of generally arid conditions, but are well adapted strand plants. *G. hirsutum* race *yucatenense* is confined to the coastal strip along the northern shore of the Yucatan peninsula which is constantly exposed to the prevailing northeast trade winds. This coast is windswept

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and barren, except for a low xerophytic scrub of which *yucatanense* forms a dominant part. The plants are extremely prostrate: in places they extend right down to the high tidal zone, and must constantly be exposed to salt spray. In culture, twice-daily spraying with sea water over a period of three weeks failed to produce any foliar burning, shrivelling or other deleterious symptoms. A wild form of *G. hirsutum* race *marie galante* was collected by Mr. J. H. Kempton on the island of Piritu Adentro off the coast of Venezuela. According to his description this wild cotton is found as "almost the dominant vegetation of Piritu Adentro and Piritu Afuera, low coral islands constantly swept by salt spray." A wild form of *G. hirsutum* race *punctatum* was collected by Hutchinson (1944) on a "windswept coastal plain" in Puerto Rico. According to Dr. C. M. Rick (personal communication) the wild cottons of the Galapagos Islands, *G. klotzschianum* and *G. barbadense* var. *darwinii* are usually restricted to the arid coastal zones and often occur within ten feet of high tide mark. *G. armourianum* and *G. harknessii* both have leaves with the coriaceous texture which Boyce (1951, 1954) has shown to be associated with salt-spray tolerance in several other coastal ecotypes. Finally the experiments of Lunt and Nelson (1951) have shown that cultivated *hirsutum* cottons can partially replace potassium by sodium in their metabolic requirements, a capacity which is shared by other cultivated plants which probably evolved from wild coastal ancestors (e.g., beets, Swiss Chard, turnips, celery).

When plants are known to occupy coastal and insular habitats, it is natural to consider the possibility that their seeds may be dispersed by ocean currents. Clearly there are four main factors involved in oceanic dispersal:

(1) Opportunity for viable seeds to be deposited in the sea;
(2) Capacity of the seeds to float on sea water;
(3) Capacity of the seeds to remain viable under prolonged immersion in sea water;
(4) Ability to germinate and establish seedlings near to high water mark.

Since the species of Gossypium under consideration all exist as coastal ecotypes, Factors 1 and 4 require no further discussion, and attention will be confined to Factors 2 and 3, namely, buoyancy and salt water tolerance respectively.

BUOYANCY

During his extensive studies of the buoyancy of the seeds and fruits of littoral species, Guppy (1906) rejected the possibility that the seeds of the Hawaiian and Tahitian wild cottons could be dispersed by ocean currents, because his experiments showed that they sank in sea water within one or two weeks. He also noted in other genera that the time of sinking was often associated either with decomposition, or with germination followed by decomposition, of the seeds. As will be shown elsewhere in this paper, the latter observation is not generally applicable to cotton seeds.

Over intermittent periods, and as opportunity offered, the buoyancies of the seeds of various species of Gossypium have been tested in this laboratory. The method employed is the simple one of placing the seeds to be tested in tall glass cylinders filled with sea water (or with 3.5 per cent sodium chloride solution which has approximately the same density) and noting the time elapsing before the seeds fall to the bottom of the cylinder. The cylinders are shaken daily to ensure thorough surface wetting of the seeds. The following species have been tested: *G. arboreum, G. hirsutum, G. barbadense, G. thurberi, G. raimondii, G. klotzschianum* var *davidsonii, G. armourianum* and *G. anomalum.* The mature seeds of all these species sink immediately in sea water if their seed fibers are removed previously by acid treatment. Without previous acid treatment, the floating time apparently depends on the amount, fineness and surface properties of the fibers borne on the seeds. Thus the seeds of *thurberi, davidsonii* and *armourianum* which have short and sparse or closely matted fibers sink immediately or within a few hours; *anomalum* seeds which have a dense cover of longer fibers remain floating from one to two weeks; cultivated *hirsutum* (Upland) seeds with a full cover of true lint can float for as long as three weeks. Seeds of the wild Galapagos cottons (*G. barbadense* var. *darwinii*) which have extremely fine and slippery-surfaced fibers have proved to be more buoyant than any others so far tested. One sample from Charles Island remained floating for 10 weeks with no apparent tendency to sink, at which time the test was discontinued. Apparently the finer linted types trap air bubbles to a greater extent than the coarser linted types; this may be a major factor in keeping the seeds afloat.

Since the mature capsules (bolls) of all species of Gossypium have light woody walls with papery septa, the possibility that seeds might be transported in the boll was also considered. The bolls of the cultivated species flare widely open at maturity, and it was found, rather unexpectedly, that they soon became water-soaked and often sank more rapidly than the seeds they contained. The mature capsules of many of the wild species (e.g., *davidsonii, thurberi*) remain partially closed, the seeds escaping through apical slits between the carpels. Such capsules can remain afloat for a longer period, presumably because of air bubbles trapped inside them. None floated for longer than two weeks.

Green bolls which had almost attained full term but were still undehisced were found to be more buoyant than mature bolls, and they often floated for about three weeks. Since it is usually possible to extract seeds from bolls in this stage of development and to germinate them successfully after a preliminary period of drying, the oceanic transport of seeds enclosed in intact bolls is a possibility for consideration.

These preliminary observations suggest that seeds of several species of Gossypium are capable of remaining afloat on sea water, either enclosed in undehisced bolls (wild species) or as free seeds (linted, cultivated species)

for a period of three weeks. Certain types which have particularly fine fibers (e.g., *darwinii*) are capable of floating at least ten weeks and probably for a much longer period. Thus without having to postulate assistance from natural rafts, a current moving with the moderate velocity of a half mile per hour could carry the seeds of several cotton species for a distance of 250 miles, and at least 800 miles in the case of *darwinii.*

SALT WATER TOLERANCE

Most wild species and all wild forms of the cultivated species of Gossypium have seeds with hard coats. They do not germinate readily, either when newly harvested or after prolonged storage. In experimental work it is a common practice either to chip the seed coats or alternatively to give them a preliminary hot water treatment. After either of these pre-treatments they absorb water within a few hours and germination begins. In nature, presumably, the seed coats are subjected to chemical or physical reactions in the soil which produce an effect similar to that obtained by pre-treatment in the laboratory. It is known that the seeds may remain dormant in the soil over several seasons. Modern cultivated cottons, on the other hand, have seeds with thin coats. They absorb water readily and germinate promptly. This characteristic has undoubtedly developed through unconscious selection accompanying the breeding of annual cottons. Prompt germination is essential for a plant which has to complete its life cycle within the limits of a single growing season. Perennial cottons which grow as "dooryard" forms in frost-free areas often have seeds with hard coats.

Modern cultivated cottons (*G. arboreum, G. hirsutum* and *G. barbadense*) with thin seed coats, were found to absorb sea water readily. They showed no sign of germination while exposed to sea water and rapidly lost their viability. This was tested by washing the seeds thoroughly in fresh water and transferring them to germinating pads. The capacity to germinate was greatly reduced after one week's exposure to sea water, and lost entirely after two week's exposure. Further attention was paid, therefore, to the seeds of wild forms which usually have hard coats. Since, at the time, only *davidsonii* seeds were available for study in sufficient numbers, they were used in the first tests.

(1) *G. klotzschianum* var. *davidsonii.* This wild species occurs as a coastal and insular form in Lower California (see table 1). It is closely related to *G. klotzschianum* sensu strictu which is endemic to the Galapagos Islands.

A sample of fully matured seeds with fibers intact was placed in a glass tank of sea water (one gallon capacity). Most of the seeds sank immediately to the bottom of the tank; the remainder sank within a few hours. During the test the temperature at the bottom of the tank fluctuated between 23° and 26°C. Losses due to evaporation were replaced by adding distilled water at intervals to maintain a constant level. At 14-day intervals, samples

of ten seeds were removed from the tank, dried for 24 hours at 30°C, and then washed thoroughly in tap water. The seed coats were then chipped, and the seeds placed on germinating pads. The test was continued for eight weeks.

A second test of a similar nature was carried out, but substituting a procedure of alternate wetting and drying for the continuous immersion used in the first test. The seeds were soaked daily for 30 minutes, then dried for the remainder of 24 hours; the cycle being repeated over the whole eight-week period.

The results of both tests are given in table 2. They do not differ appreciably, and no detectable loss of viability occurred in either test.

TABLE 2

THE EFFECTS OF EXPOSURE TO SEA-WATER ON THE VIABILITY OF SEEDS OF *G. KLOTZSCHIANUM* VAR. *DAVIDSONII*. THE FIGURES REPRESENT THE NUMBERS OF VIABLE SEEDS IN SAMPLES OF TEN SEEDS TESTED.

	Number of weeks exposed				
	0	2	4	6	8
Continuous immersion	10	9	7	8	10
Alternate wetting and drying	10	10	8	9	10

(2) *Other species:* In a second series of experiments, the salt water tolerance of seeds of the following coastal forms (see table 1) was examined:

G. armourianum
G. klotzschianum var. *davidsonii*
G. hirsutum race *marie galante* (2 forms)
G. barbadense var. *darwinii*

Seeds of the following inland species were also included in the tests for comparative purposes:

G. thurberi Tod. wild "D group" species from Arizona
G. raimondii Ulb. wild "D group" species from Peru
G. anomalum Wawra & Peyr. Wild "B group" species from Sahara desert region

The two forms of *marie galante* included in the tests were (1) a wild form with sparse lint collected on the island of Piritu Adentro by Mr. J. H. Kempton, which has been mentioned previously and (2) a dooryard form collected in the port of San José, Guatemala, which has copious white lint and a hard seed coat. The *darwinii* sample was collected on Charles Island (Galapagos) by Dr. C. M. Rick.

In this series of tests, the seeds with fibers intact were subjected to continuous immersion for a period of 10 weeks. The experimental procedure differed from that described previously in that a 3.5 per cent solution of sodium chloride was substituted for natural sea water which was not readily available. Also the glass tank was replaced by a series of 500 cc glass

cylinders, which were covered to prevent evaporation. The cylinders were shaken at daily intervals. The inclusion of *davidsonii* seeds in the tests served as a check on the changed procedure.

From the results presented in table 3 the conclusion may be drawn that the seeds of all the coastal forms which were tested, with the exception of *G. armourianum,* were remarkably tolerant to salt water immersion. At the end of the 10-week period the seeds of *davidsonii, darwinii* and the wild form of *marie galante* showed no detectable loss in viability. Seeds of the dooryard form of *marie galante* remained fully viable for four weeks, but showed some decline at the end of the 10-week period. The *armourianum* seeds were completely inviable after two weeks' immersion. (These were the only thin coated seeds included in the tests.)

TABLE 3

THE EFFECTS OF CONTINUOUS IMMERSION IN 3.5% NaCl SOLUTION ON SEED VIABILITY OF COASTAL AND INLAND FORMS OF GOSSYPIUM. THE FIGURES REPRESENT THE NUMBERS OF VIABLE SEEDS IN SAMPLES OF TEN SEEDS TESTED.

	Number of weeks exposed			
	0	2	4	10
Coastal				
davidsonii	5	7	6	8
armourianum	9	0	..	..
marie galante (wild)	4	8	8	8
marie galante (dooryard)	9	..	10	6
darwinii	10	..	9	9
Inland				
thurberi	9	5	0	..
raimondii	8	..	4	4
anomalum	4	4	3*	0

*Slow germination.

Of the inland forms tested, only *raimondii* seeds survived the 10-week immersion, and they showed an apparent partial loss of viability. The seeds of *anomalum,* which have extremely hard coats, showed weakened viability at the end of four weeks, which suggests that a hard seed coat, though important, is probably not the only factor determining salt water tolerance.

DISCUSSION

The experimental data show that the seeds of several coastal ecotypes of Gossypium which have hard seed coats are able to resist long exposure to sea water without losing their viability. The upper limit of their tolerance is not known, but it certainly persists for several months and probably for longer periods. The limiting factors to their dispersal by ocean currents are, therefore, the direction and velocity of the particular current and the buoyancy of the seeds or capsules which it carries. In the case of several species an estimated "cruising range" of 200 miles is probably fairly conservative. This is sufficient to provide opportunity for the transport of seeds along shore lines, from mainland to off-shore islands, (e.g., the Gulf

of California) and from island to island in certain groups (e.g., the Antilles, Bahamas, Florida Keys and the Galapagos archipelago). It is clearly not sufficient to account for the initial colonization by wild forms of true oceanic islands like the Galapagos and Polynesian Islands unless the further assumption is made that their seeds could form part of floating masses of vegetation such as have been described in "El Niño" years off the Peruvian coast (Murphy, 1926).

In the particular case of *darwinii*, it seems quite evident that transport over much longer distances would be possible without invoking the aid of "natural rafts." It has been shown that their seeds with attached fibers can remain floating for a minimum of 10 weeks without showing any apparent loss of buoyancy. Again adopting a conservative estimate, a cruising range of about 800 miles would be entirely possible. The nearest mainland rela-

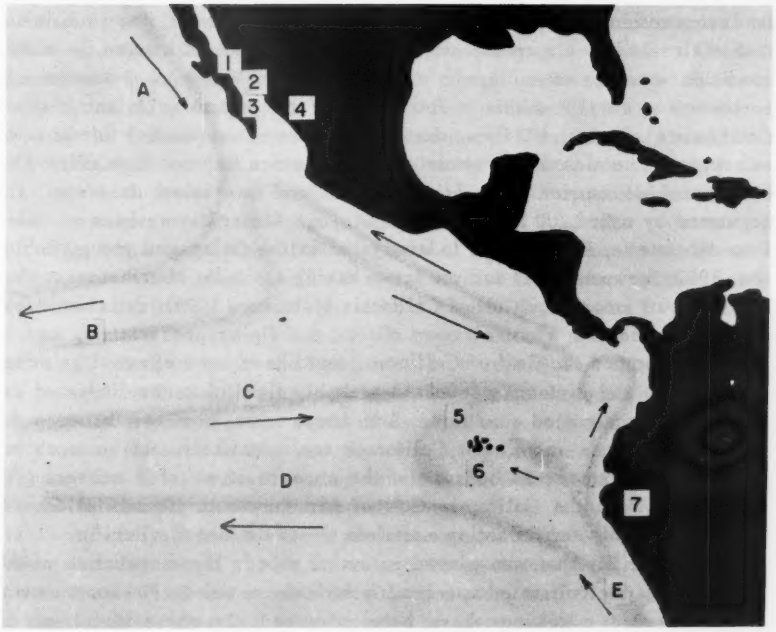

FIGURE 1. The Pacific coastline of tropical America, showing locations of coastal and insular forms of Gossypium, in relation to ocean currents. The species are indicated by figures; the currents by letters as follows:

Species

(1) *armourianum*
(2) *harknessii*
(3) *davidsonii*
(4) *aridum*
(5) *klotzschianum*
(6) *darwinii*
(7) *barbadense* ("Tumbes wild")

Currents

(A) California Current
(B) North Equatorial Current
(C) Equatorial Counter Current
(D) South Equatorial Current
(E) Humboldt (Peru) Current

tives of the *darwinii* cottons are in all probability the wild *barbadense* types which are found in the Tumbes region of northern Peru (Svenson, cited Stebbins, 1947; Hutchinson *et al.*, 1947; Kearney, 1952). The Galapagos Islands are less than 800 miles from the Tumbes coast and both lie in the path of the Humboldt Current (figure 1). If the *darwinii* cottons reached the Galapagos Islands without the aid of man, it is clear that their seeds *could* have floated there on the Humboldt Current, which has a surface movement of about one mile per hour where it turns westward from the Peruvian coast (Murphy, 1923).

The lack of spinnable seed fibers or other recorded useful properties makes it evident that man could have played no direct part in the distribution of those wild species of Gossypium which occur as insular forms (e.g., *armourianum, harknessii, klotzschianum* and its variety *davidsonii* which are listed in table 1). Thus unless one is prepared to build hypothetical land connections across every intervening body of water, the probability that their seeds were transported by ocean current and arrived in viable condition must be considered. The islands on which *armourianum* and *harknessii* occur lie within a few miles of the shoreline in the Gulf of California (Johnston, 1924), so that the absence of any marked tolerance to salt water immersion in the seeds of these species may not have offered an insurmountable barrier. But *klotzschianum* and its variety *davidsonii* are separated by over 1200 miles of open sea; the former having been recorded from Albermarle, Bindloe and Indefatigable in the Galapagos group (Robinson, 1902; Stewart, 1911) and the latter having a similar distribution to that of *harknessii* around the Gulf of California (Johnston, 1924). It is likely that the distribution of *klotzschianum* within the Galapagos Islands, and of *davidsonii* around the Gulf of California could have been effected by ocean currents, as the distances involved probably lie within the limits of the buoyancy of their seed capsules. Any direct route, however, between the Galapagos Islands and Lower California seems unlikely, not so much because of the distance as because of the absence of suitable currents (see figure 1). Both the California Current and the South Equatorial Current would presumably carry floating materials westward into the Pacific.

The possibility that man played an active role in the distribution of the wild forms of the cultivated species *(G. barbadense* and *G. hirsutum)* cannot be disregarded. Reasons have been advanced elsewhere (Stephens, in press) for believing that these forms originated as escapes from former cultivations. This would in no way imply that their present insular and coastal habitats mark the sites of previous settled cultures. The early use of cotton in the New World may have been associated with the needs of fishing communities. The archaeological discoveries in the Huaca Prieta site in Peru (Bird, 1948) suggest that the earliest use of cotton thread was in the making of fishing lines and nets. The Caribs, too, in the West Indies carried cotton with them on their fishing trips. I am grateful to the ethnologist, Mr. Douglas Taylor, Dominica, B. W. I. for bringing to my attention the following quotation from Raymond Breton's Carib-French Dictionary (1609-1679):

The Savages (Caribs) cannot do without cotton, whether for their beds, for threading their trinkets, for putting feathers to their arrows, and adjusting their green wood, or the stingray tails that serve in the place of points. For that reason, when they go to the Saints fishing, or to Marie Galante to collect crabs, they make some clearings there to plant cotton. You see very few Savages who do not always have a little ball of this thread in their basket.

Marie Galante and The Saints are small islands lying between Dominica and Guadaloupe in the Lesser Antilles.

This quotation is of considerable importance since it provides a reasonable explanation for the presence of wild forms of the cultivated species in small islands and as the components of coastal vegetation. The establishment of a few plants around temporary fishing camps could easily have provided the focal points for subsequent seed dispersal by natural means, particularly ocean currents. Of major interest in this connection is the recent discovery by Heyerdahl and Skjolsvold (1956) of archaeological remains (pre-Spanish pottery) on three of the Galapagos Islands: Charles, James and Indefatigable. The sites are thought to mark the positions of temporary fishing camps, and the suggestion is made that the *darwinii* cottons of the Galapagos may have been introduced intentionally or accidentally by early fisher-folk. In view of the known practices of the West Indian Caribs which have been quoted above, it seems very probable that the early visitors to the Galapagos Islands followed a similar procedure of planting cotton near their fishing grounds. On the other hand, *darwinii* cottons are established on *all* the islands which have been systematically collected, including small islets like Gardner, Jervis and Eden; and it seems unlikely that every island would have furnished a site for a separate planting. More probably the seeds would be spread from island to island by natural means, following relatively few human introductions. It has been shown earlier that the seeds of *darwinii* have sufficient buoyancy and salt water tolerance to complete the colonization of the whole archipelago. Whether the first *darwinii* cottons reached the Galapagos Islands via the Humboldt current, or were carried there by man, *it is probable that their seeds were fully linted and hence probably originated from cultivated plants.* Otherwise they would neither have been buoyant enough for oceanic transport nor useful enough to have been carried along on a fishing expedition. In short, the *darwinii* cottons are probably a recent addition to the Galapagos flora.

SUMMARY

Several wild species, and wild forms of the cultivated species of Gossypium occupy coastal or insular habitats. The seeds and/or capsules of these forms have a limited buoyancy in sea water, which is, however, sufficient to provide opportunity for dispersal along shore-lines, to off-shore islands, and from island to island in certain cases. Seeds of coastal and insular forms usually have hard seed coats and can remain viable after long periods of immersion in sea water. The seeds of *Gossypium barbadense* var. *dar-*

winii have both sufficient buoyancy and salt water tolerance to have enabled them to reach the Galapagos Islands from the mainland of South America. The possible roles of man and ocean currents in the dispersal of these cottons is discussed.

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GENIC CONVERSION IN OENOTHERA?
A Critical Review*

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During the past years a number of geneticists have used Winkler's term "conversion of genes," meaning originally a method of mutual influence of alleles upon each other in explanation of crossing over without segmental interchange. Winkler had pointed out that tetrad analysis might lead to a proof of conversion. The phenomena which led to an exhumation of this term are not completely alike, but have in common that they do not fit into the classic conception of segregation and crossing over. For a discussion of the new facts and their explanation see the recent symposia in Cold Spring Harbor and Baltimore (1957) especially papers and discussion remarks by Mitchell, Lindegren, Bonner, Roman, Glass, Catcheside, and others. It seems that many American geneticists have overlooked the fact that O. Renner during the last twenty years has described and analyzed phenomena found in Oenothera crosses which clearly belong to the group of facts just mentioned and which he interpreted from the beginning as examples of Winkler's conversion of the genes, without trying to give a specific cytological or biochemical meaning to the phenomenon named "conversion," while insisting simply upon the difference from the facts of classical genetics. In some respects the facts go further than the recent work, mostly done with lower plants, while trailing behind them in other respects like the use of markers, which is almost impossible in Oenothera. Therefore, attention should be called to them and a tentative explanation proposed based upon recent findings in Drosophila. Simultaneously with Renner, Oehlkers worked on the same problem and presented a different explanation, as we shall see below.

The experiments were performed with a series of Oenothera forms, homozygous and complex-heterozygous ones; the latter furnishing the decisive facts. The first example, which Renner still regards as the "paradigma of the anomaly," was the cross *Oe.* (atrovirens × biennis) *pictirubata.* (We follow here throughout Renner's classic terminology for the complex heterozygous and normal species and their offspring.) The work relates to the cruciata character, which consists of sepaloidy, i.e., change of petals into sepals. It had already been found that the cruciata character behaves as a single Mendelian recessive. There is no direct proof for this, but it is shown in crosses between a cruciate complex heterozygote and a homozygous form; further by analogy with cruciata in the nearly related Epilobium. There seems to be still doubt about the chromosome in which *cr* is located.

*For Otto Renner on the occasion of his 75th birthday.

In his last paper Renner assumes a location in chromosome 5.6. Thus all work on the subject assumes that *Cr cr* are mendelizing alleles. But it might be stated at the outset that there is no direct proof of it, though it is clearly necessary to work with such an assumption.

It seems that in different lines of cruciata different alleles are present. Crosses between such lines are all cruciata but the degree of sepaloidy (which is the cruciata character) may be different in different crosses. The heterozygote *Cr cr* is not constant, as de Vries already knew and Oehlkers analyzed in detail. A few combinations show simple dominance of *Cr* but others give a very variable F_1 with all transitions from the normal to the cruciate type, including the extremes. Even in a single plant all these types may be found. This is, however, not a case of modification (environmental or via dominance modifiers). Selfed normal plants or individual flowers produce essentially their like and so do cruciate flowers. Thus Oehlkers concluded that mutability of the "labile gene" type is involved. Renner showed now by backcrossing cruciate F_1 both with cruciate and normal forms that the F_1 cruciates behaved like genetically *cr cr* forms and continued to breed thus. In the same way, it was shown that normal F_1 flowers could also breed as *Cr Cr* homozygotes. In both cases Renner thought that somatic crossing over could not explain the facts. Of special interest are now the different grades of intermediates in F_1. Actually, these can be selected for, as shown by Oehlkers and others. This is, of course, in favor of the presence of multiple modifiers for dominance (or penetrance and expressivity). But Renner proposed (1937) to consider the possibility that the "mutation" of *Cr* to *cr* and vice versa in the hybrid could occur in different steps. Thus Renner goes back to Winkler's "monogenic conversion," which means that, in the heterozygote, one allele can transform its partner into its own type, even with intermediate conditions as a possibility, while Oehlkers considered the facts as another example of so-called "mutable loci." Renner claims in favor of his side the fact that the "conversion" takes place only in the heterozygote, while both homozygotes are completely constant. Another important point made by Renner is that the phenomena described occur in all lines tried in manifold combinations It would be surprising if sets of modifiers for dominance were always present identically whatever the origin of the *Cr* and *cr* parents.

Later Renner (1942) considered a number of backcrosses as among the best proofs of his interpretation. Examples are:

1) (*cr*-atrovirens × *Cr*-biennis) *cr-pictirubata* × *Cr-biennis* = all *Cr-pictirubata*
2) *cr*-atrovirens × (*cr*-atrov. × *Cr-biennis*)*Cr-pictirubata* = all *cr-pictirubata*

Renner says: if a cruciate *pictirubata* (i.e., containing the complexes rubens and pingens) were an individual in which a specially weak *Cr* in rubens is recessive to *cr* in pingens, one would expect that the backcross *cr-pictirubata* × *Cr-biennis* produce the same number of cruciate offspring as the original cross atrovirens × biennis. Further, if the cruciate *pictirubata* were a plant in which a specially strong *cr* dominated *Cr* in rubens the backcross *cr-*

pictirubata × *Cr-biennis* should contain more cruciates than F_1 does. But all offspring are normal. From this and other similar results the conclusion is drawn that *cr* in pingens of the cruciate *pictirubata* cannot be in the same condition as in atrovirens before crossing. "If it comes through in presence of *Cr* of rubens and has adapted the latter to its own status, it is nevertheless shaken by its meeting with *Cr* and, when meeting a 'fresh' *Cr* from rubens, will always be converted; and even the *Cr* from rubens is not completely unchanged after meeting with *cr* when this had been victor in the competition for conversion, e.g., if the rubens of a normal *pictirubata* meets fresh *cr*-pingens in the cross with *cr*-atrovirens female it succumbs almost always." Further: "Which of both partners is the more labile one after conversion, the converted one as one should think, or the converting one, cannot be decided with certainty. We know only that both are more labile after conversion than they were in the original lines." Further: "Whereas the selfed cruciate *pictirubata* returns to normal and the normal *flexirubata* similarly to cruciate we may assume safely that it is the first converted gene which returns to the original state, without being pushed by a new hybridization. The *cr cr-flexirubata* is then at once homozygous. If cruciate *flexirubata* segregates from the normal one it appears as a *cr*-homozygote which means that the reverted *cr* from flectens had converted also *Cr* from rubens to *cr*."

Another group of facts, which Renner considers proofs for conversion, relates to crosses with an *Oe. Lamarckiana* line with almost cruciate character described as subcruciata, (Renner and Sensenhauer, 1942). If this is crossed with the extremely cruciate atrovirens the hybrids are extremely cruciate; but if subcruciate is crossed to normal, F_1 is normal. But the same subcruciata if crossed to a normal hybrid *Oe.* (atrovirens × biennis) *pictirubata* (i.e., the "converted" hybrid) produces also normals and if the cross is made with cruciates of the same hybrid all offspring are cruciate, just as if both hybrids had been *Cr Cr* viz. *cr cr*, i.e., the normal *pictirubata* had also in the pingens complex *Cr* and not *cr* and the cruciate hybrid had also in the rubens complex *cr*, not *Cr*. This is considered a strong proof for conversion. A test with selfing of *pictirubata* had also shown that the normals were very stable while the cruciates were more labile, i.e., "mutated" back in part to *Cr*.

In a recent paper Renner (1957) presents further similar material which in his opinion can only be explained by conversion in the heterozygote. Here are some examples:

1. *Oe. Lamarckiana subcruciata* has a less extreme cruciata type, as mentioned before. When selected, it segregated for 18 generations some intermediates and normals. The latter selfed bred true. After 18 generations *subcruciata* bred true and was completely recessive. The extracted normals (ex cruciata) are dominant over *subcruciata* and F_2 consists almost completely of normals. A backcross of F_1 with a recessive *cr cr* blandina gives the twin hybrids laeta and velutina both normal; while the same *cr*-blandina crossed with *Lam. subcruciata* produces only cruciate laeta and

velutina. Thus in the backcrosses the combinations *cr*-gaudens · *Cr*-velans or *cr*-velans · *Cr*-gaudens have been converted into *Cr*-gaudens · *Cr*-velans!

2. Similar are the results of crosses of *Cr-biennis* and *Hookeri* with *L. subcruciata.* If F_1 albilaeta and albivelutina are crossed to reconstitute the *Lamarckiana* the overwhelming majority is normal, i.e., the *cr* of gaudens and velans had become *Cr.* There are indeed a few cruciates (20: 238), to which fact Renner remarks that it only confirms the old experience that in conversion a certain amount of retaining of the original status occurs.

3. A parallel cross to the last one uses *biennis* cruciata (not the normal b.). A combination of the resulting twins into *Lamarckiana* produces only cruciates resembling *subcruciata* (not the extreme *biennis* cruciata). According to Renner this means that the *subcruciata cr* is not converted towards the extreme *cr.*

4. Crosses of normal with cruciate *biennis* gives normals, intermediates and cruciates. Normal F_1 selfed produces normals and cruciates and so does the backcross of cruciate F_1 with normals. This could be apparent segregation by crossing over (no free chromosomes here). But selfed cruciate or intermediate F_1 produce only cruciates. F_1 normals × F_1 cruciates produce also only cruciates. Further, F_1 cruciates × *Lam. subcruciata* give almost only cruciates, i.e., according to Renner *Cr* (from rubens) has been —at least almost—converted to *cr.*

5. Extensive data are presented for crosses between *biennis* and ordinary *Lamarckiana,* which are more complicated because interactions with the simultaneously present alleles for flower size *Co-co,* are involved. In F_1 *biennis cruciata* × *Lamarckiana,* the twins albilaeta and albivelutina are in the majority normal, but also a variable number of cruciates appear. F_2 selfed from normals segregates into approximately one-half of each type, while F_2 from cruciates is predominantly cruciate. In the first case also intermediates appear. Flower size segregates typically. (Actually this is no segregation but 50 per cent crossing over for a locus at the chromosomal end where it is attached to the next one. We are dealing with a chain of 14 which does not permit genuine segregation.) The result of breeding from cruciates is said to show that conversion produced *cr*-homozygotes in which, however, *cr* may revert to *Cr.* The result of breeding from normals is said to show belated conversion of *Cr* to *cr.*

6. Complicated results occur when the twins albilaeta and albivelutina are crossed together (which reconstitutes *Lamarckiana*). Normal twins produce normal *Lamarckiana*; cruciate twins produce about equal numbers of normals and cruciates. Of the cruciates, those which have small flowers breed true, but large flowered cruciates produce mostly normals. These secondary normals were tested and turned out to be much less dominant over *cr* than usual. The *cr* in the small flowered cruciate secondary *Lamarckiana* is less efficient than that of the original *biennis* cruciata. Tested with normal *biennis* the offspring is all normal. Mating these normal twins produces *Lamarckiana*; the majority of the offspring is normal. This means to Renner that the *cr*-gaudens and velans, after having been heterozygous

with *Cr*-albicans, have in part reverted to *Cr*. In all the cases of selfing the flower size "segregates" 3:1 which, however, cannot be real segregation as no free chromosomes are present, but must be a 50 per cent crossing over as already pointed out. But *Cr-cr* "segregate" in very irregular numbers and therefore this cannot be based upon a comparable crossing over. (Renner considers a 50 per cent crossing over found by Oehlkers to be a chance result. The only other locus behaving like *Co* is *Br-br*.)

Reviewing this large body of facts we pointed out for the individual sets of experiments how Renner accounts for the unusual features by the theory of genic conversion. In dealing with a material like Oenothera one has, of course, to be prepared for features which are not found in other genetic material. (Let us think of such facts—apart from the basic and generally known consequences of complex-heterozygosis and segmental interchange —as a 3:1 segregation without free chromosomes and therefore due to 50 per cent crossing over (for *Co-co*).) The abnormal behavior of the cruciate locus in the reported experiments and also the variants of abnormality described are therefore a priori under suspicion of belonging to the group of oddities connected with the unusual chromosomal behavior, rather than exemplifying a strange genetic principle. But Renner, who certainly knows Oenothera better than anybody else, points out emphatically that the hereditary behavior of the cruciates cannot be based upon the standard features of the Oenothera case but must be understood in terms of a general genetic principle which must be different from that underlying classical genetics. He sees the solution in Winkler's conversion, though he actually uses only this term without giving it any other meaning than something different from classic genic behavior.

Passing in review the remarkable facts we notice that in many cases the simple conversion in the heterozygote does not explain the facts. Additional assumptions must be introduced, some of which are difficult to visualize, e.g., the assumption introduced for the backcrosses that converted *Cr* and *cr* are more labile than the original loci and are therefore reconverted. Another such assumption is that the possibility of selection of intermediates is not the result of selection of modifiers (or, according to Oehlkers, of multiple alleles) but the expression of stepwise conversion. Also strange linkage of conversion behavior with the "segregation" for flower size is difficult to understand (loci in different chromosomes). Thus, altogether we gain the impression that indeed facts have been found which do not fit into the standard concepts of classic genetics; further that some of the abnormal features being restricted to complex heterozygotes without free chromosome pairs, must somehow be connected with the complex-heterozygote condition of the material;[1] further that some quantitative element is involved in the details of the phenomenon. But it is difficult to be-

[1] But O. Renner kindly informs me that a paper is in press on crosses of cruciate biennis with homozygous blandina from *Lamarckiana*. He produced a cruciate with the factor *sp* in a free chromosome pair (5.6). Crossed with normal blandina he obtained partial "conversion" of *sp* into *Sp* in the *Sp sp*-blandina.

come convinced that there is no other explanation left but the idea of genic conversion, working in both directions and back again, shaking in different ways the stability of the genes and working also sometimes in steps. Actually, Renner uses the term conversion for what it is worth leaving it to the future to endow it with a specific cytological or biochemical meaning.

A supplementary set of facts is due to the work of Oehlkers (1930 ff.). Oehlkers, who worked extensively on the problem and partly with the same material (*Oe. biennis* and *Lamarckiana cruciata*) reached originally a different interpretation, though he did not yet know the part of the facts which Renner presented more recently, and though he emphasized repeatedly that his factual results, as far as they go, are identical with Renner's. The real difference of procedure is that Renner emphasizes the extremes, normal and cruciate (also subcruciate), while Oehlkers was fascinated by the series of grades between the extremes and studied these quantitatively. Thus let us first follow Oehlkers' procedure and conclusions, some of which were mentioned shortly before.

It should be repeated first that both authors consider it a fact that *Cr* and *cr* are a single pair of alleles. This is not easy to prove in Oenothera as most of the work is done with complex-heterozygotes without free chromosome pairs and without much use of markers and of course with lethality of the homozygotes. It is known that in the nearly related Epilobium *Cr cr* are a typical pair of alleles, but both authors consider the indirect signs of simple allelism in Oenothera also to be satisfactory. (They consist mainly in the results of crosses with homozygous forms.)

Another point, which was already mentioned in connection with Renner's work has to do with crossing over. In the complex-heterozygotes crossing over is possible, though one should expect complications. The facts have been studied in detail by Renner, Emerson and Sturtevant and others (see Renner's review, 1942). Without going into details one can say that crossing over from one complex into the other has been found for most alleles. But as a rule only a small crossover value is found. An exception is *Co co* (flower size) and *Br br* (length of style) which show a free segregation which, in view of its occurrence in a chain of 14 chromosomes must mean a 50 per cent crossing over. Oehlkers described a similar situation for *Cr cr* and even a 9:3:3:1 segregation (i.e., 50 per cent crossovers) for *Br* and *Cr* simultaneously. But Renner considers this to be a chance result (for *Cr*) due to the complications of the process of conversion and it seems that Oehlkers has joined him since.

Oehlkers uses in his work a detailed classification of the cruciate character. Being sepaloidy, it means transformation of petals into sepals, a change which can be complete, or, with all transitions (by the way, comparable to homoeotic mutants in Drosophila), between the extremes, affect only a part of a single petal. Thus he can distinguish seven classes of the cruciate effect: (1) normals, (2) slightly abnormals, (3) low grade defectives, (4) largely defectives, (5) almost cruciates, (6) very cruciates, (7) completely cruciates (sepaloids). The decisive first fact is that selec-

tion for these types is possible (as had been known before). These types must therefore be genetically controlled, which means either by modifier systems as in many classic cases or by a series of multiple alleles. Oehlkers attacked this problem by crossing the different grades of cruciates with a standard *biennis* cruciata which had shown in crosses with a homozygous normal *bookeri* that both complexes (albicans, rubens) are equally cruciate. He found the results of such test crosses always constant in regard to the grade of sepaloidy in all combinations made and concluded therefore that this indicated genotypic differences between cruciate alleles in the different complexes tested, namely different potencies (valencies, quantities) of the individual multiple *cr*-alleles. (This parallels, of course, the method which I had used for measuring the potencies of sex-determiners in Lymantria.) Thus he could distinguish four grades (isoalleles in today's terminology) within the dominant normals and five grades within the recessive cruciates, namely:

Allele	Complexes
Cr_1	*L. Hookeri*
Cr_2	albicans and flavens from 9-suaveolens
Cr_3	albicans from *biennis*
Cr_4	rubens from *biennis*
cr_1	albicans from *biennis* cruciata
cr_2	rubens from *biennis* cruciata
cr_3	gaudens from *Lamarckiana* cruciata
cr_4	velans from *Lamarckiana* cruciata
cr_5	albicans and rubens from *biennis* cruciata apetala

All the dominants are, in present day terminology, isoalleles, and so are the two highest recessives; only cr_{1-3} are visible intermediates. It is now assumed that all these alleles and all compounds (1 + 2, etc.) have a definite quantity. By comparing the different homozygotes and compounds in regard to the average grade of cruciate phenotypes an orderly series should be found, the members of which fall into line according to expectations. Oehlkers uses the following graphic representation (fig. 1). A vertical line contains the measures for the potencies of the cruciata loci. Class I contains the four normal isoalleles, class VII the two cruciate isoalleles. The visible intermediates occupy the zone in between, classes II-IV of increasing cruciate condition. From a group of combinations with intermediate phenotype like $cr_1 \cdot cr_2$; $cr_5 \cdot cr_1$; $cr_5 \cdot cr_2$ the location of $cr_5 \cdot cr_5$ can be calculated. On the scale the location of cr_5 is given a (gratuitous) value of 10, in order to be able to express the effects of the different combinations in concrete numbers. Thus the values $Cr_1 \cdot Cr_1 - cr_5 \cdot cr_5$ found in the figure are obtained. The test for the value of the scheme is that all the combinations measured fit into the proper place. Oehlkers' data bear out this expectation. Though it is not possible to draw direct conclusions from the phenotypes to the underlying genotype, the facts demonstrate that somehow the individual races and complexes inherit the cruciate phenotype in a definite and orderly quantitative way. One might speak of quantities,

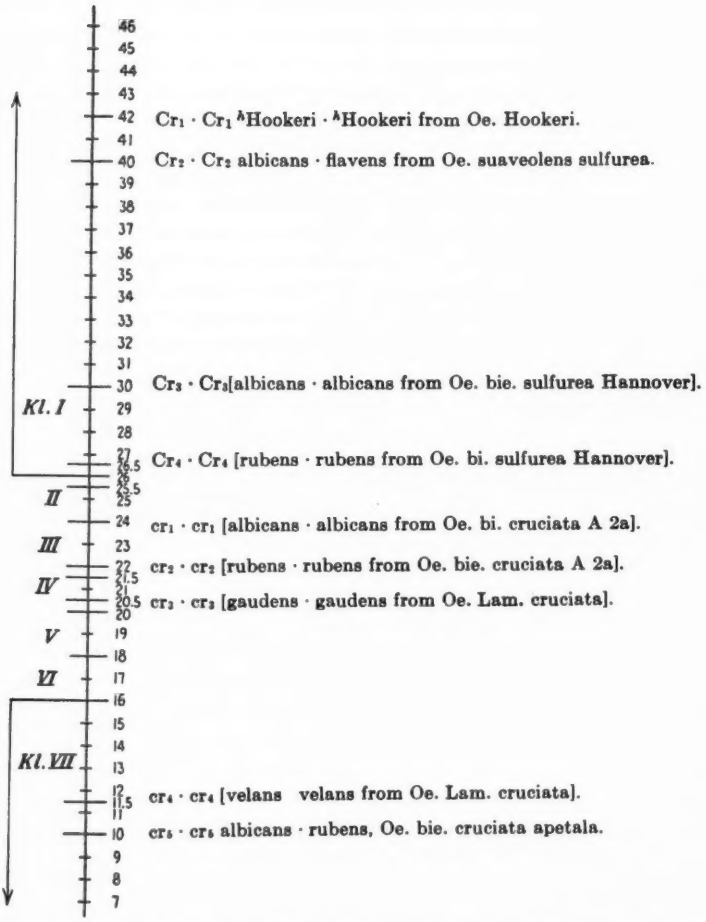

FIGURE 1. Graphic representation of the relative effects of the genes *Cr* – *cr*. In brackets, combinations that may not be obtained. Kl I–VII, phenotypic classes; *cr* 1–5, cruciate genes of increasing potency. [From Oehlkers, 1930, Ztschr. f. Bot. (Oltmann Festschr).]

potencies, or valencies of the individual alleles following the analysis in Lymantria, without prejudice to what this means cytologically or biochemically.

It is now easily demonstrated that in such a system of multiple alleles, including isoalleles, heterozygotes may show dominance of either extreme or anything in between and that such phenotypes may breed true in the balanced complex-heterozygote. Thus the facts of the *Cr cr* crosses may be understood without recourse to genic conversion, as Oehlkers and his students worked out in detail. But there are other facts in Renner's ma-

terial (as well as in some of Oehlkers') which require additional assumptions. The most important group of these is the observation that the *Cr cr* heterozygote can breed like a homozygous *Cr*, or *cr* in individual cases, as discussed above. Oehlkers, who agrees with all the factual findings of Renner, feels therefore constrained to introduce additional notions.

One possibility is that in the heterozygote *Cr cr* a change of dominance at either locus may occur which remains more or less constant; or that each of the loci and their multiple alleles may mutate, both genetically and somatically, which would make the locus *Cr* one of the "labile genes." It seems that with the introduction of mutable loci the differences between Oehlkers and Renner become largely semantic as one may describe conversion also as mutation in the heterozygote under the influence of the other allele. But there remain still differences: conversion works only in the heterozygote and may be reciprocal, which is not the case for labile loci. The details of Renner's findings which were outlined above do not fit into the facts known nowadays, after McClintock's work, for so-called "labile genes." Thus it seems that even after incorporating Oehlkers' explanation, i.e., the part relating to the multiple allelic setup, into Renner's conception as a means of explaining further detail, there remain still the decisive facts (especially homozygosis of the "converted" locus) which point out that the cruciata story must be based upon some unusual, still unexplained phenomenon, whether we call it conversion or not. An attempt at interpretation must account for these facts at least:

1. The phenomenon is limited to complex-heterozygous Oenotheras. In the nearly related Epilobium, *Cr-cr* show ordinary Mendelian behavior.
2. It works only in the heterozygote.
3. It works in both directions, *Cr* → *cr* and vice versa.
4. There is an orderly quantitative element involved, which accounts for the intermediate phenotypes and their selectability.
5. The happenings called conversion can occur at meiosis as well as at mitosis.

The question can now be raised, whether facts exist which can lead to an understanding of the Oenothera case by replacing the vague idea of conversion by a definite cytological concept. We believe that our recent work in Drosophila, incomplete as it is, provides the clues. The relevant points of this work are: the so-called mutant Bar (eyes) is known to be the effect of a tandem duplication of a small section of the X-chromosome (Bridges, Muller). A number of alleles are known, some of which are position effects of rearrangement breaks near that segment (baroids), others have not yet been cleared up cytologically (infrabar); the most important ones result from multiplication of the $+^{B}$ segment. Triplication has the effect ultrabar (double Bar) and even quadriplication has been obtained by Rapoport. In this series of 1-2-3-4 Bar segments the phenotypic effect is a decrease from normal eye size to a tiny speck of a few facets and the compounds fit into such a quantitative series of effects. Sturtevant had shown in a classic paper that the step from B to BB is produced by unequal crossing over.

This means that during a crossing over very near to the Bar locus (in the old terminology) in the B/B chromosome one B may cross into the other B chromosome unequally so that chromosomes BB and $+^B$ result. This could happen when B/B synapse asymmetrically so that a crossover break falls to the right of one and to the left of the other allele. The behavior of markers to the right and left of B bore this out in Sturtevant's experiments. Now, after the discovery of the cytological nature of B and BB = duplication and triplication of a segment unequal crossing over would mean a steplike synapsis of the segments and a crossover break between two of them.

In our new work (1957) very rare cases were found in which under the influence or control of another locus such an unequal crossing over at the Bar locus occurred in a way which might also be described as a conversion, because it did not occur in a few crossover gametes but in all gametes containing the controlling locus, (actually in almost all gametes). The heterozygote +BB/r+ (r–rudimentary 2.5 units left of B) formed gametes ½B ½rB. If one were ignorant of the cytological nature of Bar and double Bar one could say that the normal $+^B$ locus in the *r*-chromosome was converted in 100 per cent to B. The small phenotypic difference between BB and B (in terms of facet numbers) might be absent in another parallel material or so small that it is not registered so that an observer who finds such a thing in a less completely analyzed material than Drosophila, let us say for the character *Aa* with a marker *c* would describe such behavior as conversion of $\frac{+A}{ca}$ into $\frac{+A}{cA}$. Thus conversion could mean unequal crossing over in a locus which in fact (thus far invisible both cytologically and phenotypically) presents a segmental duplication, triplication, etc. If this happens, not in a rare crossover but, under the influence of a controlling locus, in all gametes (or a somatic cell) a conversionlike effect results. In our Drosophila work we pointed out that the so-called unequal crossing over might also be the equivalent of transduction of a chromosomal segment in bacteria. The controlling locus would then play the role of the phage in bacterial transduction and crossing over would not be involved at all, which would ease the explanation of 100 per cent "conversion." Unfortunately, there was no marker right of Bar in our cases, which alone would permit us to decide between unequal crossover and transduction.

It is of considerable importance that the prerequisite for such an interpretation is already available in Oenothera, though not for the *Cr*-locus. Catcheside (1947) showed that in Oenothera blandina two loci occur as duplications (*P* for red in the sepals and *S* for yellow in the petals) and that they are due to unequal crossing over. The phenotypic action involves a position effect as does the Bar action in Drosophila. Thus an application of the Drosophila facts to Oenothera is a permissible hypothesis.

There is another possibility for arriving at the same consequences. The locus which shows conversion could be one of the pseudoallelic chromosome segments which play such a role in recent genetic literature (for a critical discussion see Goldschmidt, 1955). Such a segment, which we now

know to consist of a few up to a great many subunits (Pontecorvo, Demerec, etc.) which in virus may consist even of a few nucleotides (Benzer) can be broken up by crossing over within. (See the Symposia 1957.) The effect would be the same as separation of Bar segments and therefore the same conclusions regarding conversion could be drawn. In this case it is known also that the parts of a segment act like multiple alleles in the proper combinations. If the different phenotypes of such a series could be arranged into a quantitative series, unequal 100 per cent crossing over or transduction could add to the conversion the quantitative element which Oehlkers analyzed for the cruciata character.

I do not think that it is already possible to make a detailed analysis of the Oenothera work so as to explain every single result. But it seems that one can visualize such an analysis which renounces the idea of conversion in favor of an explanation by controlling loci (transductors), complicated chromosomal segments instead of loci, unequal crossing over or transduction within the segment. All the individual typical and atypical events in Oenothera can be understood in principle on such a basis. But an analysis of the details should be very difficult in a form with complex heterozygosis and hardly any markers so that the analysis is almost completely based upon induction from the phenotype. In addition only an Oenothera worker would master the facts sufficiently to be in a position to work out a detailed hypothesis. The work of Catcheside on position effect and duplication in Oenothera might show the way. But a cytological proof can hardly be expected with Oenothera chromosomes being what they are.

SUMMARY

The remarkable facts found by Renner and Oehlkers in Oenothera crosses involving the cruciata character (calycanthemy) are reviewed and the interpretations of both authors confronted. It is shown that the facts require an unusual genetic behavior which, for Renner, is the process of genic conversion. It is discussed that recent findings in Drosophila point to the possibility that the apparent conversion is in fact unequal crossing over in a duplication, which might be controlled by a specific genetic condition comparable to *Dt* and *Ac* in maize. It should be difficult to prove any interpretation in a material like Oenothera.

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ORGAN METABOLISM IN MATURE MAMMALS AS THE PRODUCT OF ALLOMETRIC MASS AND RATE

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It is generally agreed that the relationship between metabolic rate and body weight in mature mammals may be approximated by the expression:

$$\text{Cal/day} = k \cdot W^{0.75} \text{ (Kleiber, 1947).}$$

To express metabolic rate in the form of intensity it is necessary to divide each side of the equation by the body weight:

$$\text{Cal/kg/day} = k' \cdot W^{-0.25}.$$

In the second form the metabolic rate of the intact animal is directly comparable with the metabolic rate of the excised organs, a value usually expressed in similar form (ccO_2/hr/gm or QO_2).

Krebs examined organ QO_2 in mammals ranging in size from the mouse to the horse (Krebs, 1950). His findings for liver, brain, and kidney are recorded in Table 1 together with the metabolic rates of the intact animals. It is clear that the relative differences in organ QO_2 are of smaller magnitude than the metabolic rate differences characteristic of the intact animals. He concluded that the metabolism of the organs was not adjusted to the metabolic requirements of the intact animals. Although this conclusion seems reasonable, it is not necessarily justified for the following reasons.

The animal may arbitrarily be regarded as the sum of its organs and systems. A size dependent decrease in the metabolic contribution of each organ or tissue system may be due to one or both of two factors. (1) The specific metabolic rate (QO_2) of the organ may decrease with increasing body weight. (2) The relative mass of the organ (organ weight/total weight) may decrease as a function of body weight. In either case the metabolic contribution of the organ will decrease as body weight increases. These two factors must be considered simultaneously in order to compare organ metabolism with the metabolism of the intact animal. Assuming for the moment that organ QO_2 and relative organ weight each vary as simple power functions of the body weight (as does the metabolic rate of the intact animals), a formal treatment may be given as follows:

Let:

$(ccO_2)_o$ = oxygen consumed by organ
O = weight of organ
W = total body weight
k, k', b, b' = constants

TABLE 1

	Body Wt. Kgms.	$-QO_2$			Cal/Kg./ 24 hours Whole Animal
		Brain Cortex	Kidney Cortex	Liver	
Mouse	.021	32.9	46.1	23.1	158
Rat	.21	26.3	38.2	17.2	100
G. pig	.51	27.3	31.8	13.0	82
Rabbit	1.05	28.2	34.5	11.6	60
Cat	2.75	26.9	22.7	13.2	50
Dog	15.9	21.2	27.0	11.7	34
Sheep	49.0	19.7	27.5	8.5	25
Cattle	420.	17.2	23.5	8.2	20
Horse	725.	15.7	21.5	5.4	17

From Krebs, 1950.

Then:

(1) $$\text{metabolic rate of organ} = \frac{(ccO_2)_o}{O \cdot hr} = k \cdot W^{-b}$$

(2) $$\text{relative organ weight} = \frac{O}{W} = k' \cdot W^{-b'}$$

By multiplying equation (1) times equation (2) it is possible to eliminate O (organ weight) and express *organ metabolism per unit of body weight as a function of body weight.*

(3) $$\frac{(ccO_2)_o}{O \cdot hr} \cdot \frac{O}{W} = k \cdot k' \cdot W^{-(b + b')}$$

which reduces to:

$$\frac{(ccO_2)_o}{W \cdot hr} = K \cdot W^{-(b + b')}$$

Equation (3) is in a form which more accurately represents the manner in which organ metabolism varies with body weight since it recognizes alterations both in QO_2 and relative organ mass.

Thus to evaluate organ metabolism as a function of body weight one must consider not only the variables represented in equation (1) (table 1), but recognition must also be given to the variables in equation (2) (relative organ weights). Ideally, relative organ weights should be determined for animals of the same species and adult weights as those represented in Krebs' study. Although adult weights are not identical it is possible to find values for relative organ weights for the same species for the three organs, brain, liver, and kidney. In Table 2 I have compiled from various sources relative organ weights expressed as per cent of the total body weight, thus providing the information for an analysis of equation (2).

Equations (1) and (2) are each in the general form:

$$y = k \cdot x^{-b}$$

TABLE 2

Species	Body Wt. Kgms.	(Organ Weight/Total Weight) × 100 Brain	Kidney	Liver	Source
A. mouse	0.020	1.80	...	...	Brody, 1945
A. mouse	0.025	...	1.42	...	Tab. Biol. 15, 1938
Mice*	0.026	...	...	5.0	Crile and Quiring 1940
Rat	0.25	0.80	0.84	4.8	Brody, 1945
G. pig	0.80	0.59	0.70	3.4	Brody, 1945
Rabbit	2.6	0.40	0.70	3.2	Crile and Quiring 1940
Cat**	(3.8)	(0.75)	(1.18)	(3.35)	Crile and Quiring 1940
Dog**	(10.0)	(0.75)	(0.70)	(4.20)	Brody, 1945
Sheep	52.0	0.20	0.31	1.84	Brody, 1945
Cattle	488.	0.082	0.24	1.31	Brody, 1945
Horse	600.	0.112	0.28	1.12	Brody, 1945

*An average value calculated for several species of wild mice from the data of Crile and Quiring (1940).

**Figures in parentheses (carnivores) were not plotted in the calculation of the allometric constant b.

Taking logs of each side:

$$\log y = \log k - b \cdot \log x$$

Plotting log y versus log x should result in a straight line with slope equal to –b. The information in tables 1 and 2 is plotted in logarithmic form in figures 1 and 2 respectively. The best straight lines were fitted by eye and the slopes recorded in table 3. Relative organ weights for the cat and dog were not plotted in figure 2. The organs in these species are considerably larger than in herbivores of comparable size. Judging from the data of Crile and Quiring (1940), carnivores generally have somewhat larger organs than herbivores of similar body weight. The most notable example is the brain, although liver and kidney also tend to be larger in carnivores. Using the data of Crile and Quiring it is possible to plot relative organ weights in carnivores ranging in weight from the weasel (0.169 kg.) to the polar bear (317 kg.). Although the absolute values are higher than for the animals in figure 2, similar slopes are obtained for each of the three organs. Unfortunately the carnivore data are limited to relatively few determinations for each species.

Considering the genetic heterogeneity of the animals represented both QO_2 and relative organ weights approximate power functions of the body weight. According to theory the sum of the allometric constants b and b′ should approximate the value relating metabolic rate to body weight in the intact animal (−0.25). The sums of the allometric constants recorded in table 3 are remarkably similar to the value −0.25 for whole animal metabolic rate. Liver and kidney are especially close being −0.27 and −0.23 respectively. The value for brain is somewhat higher (−0.34).

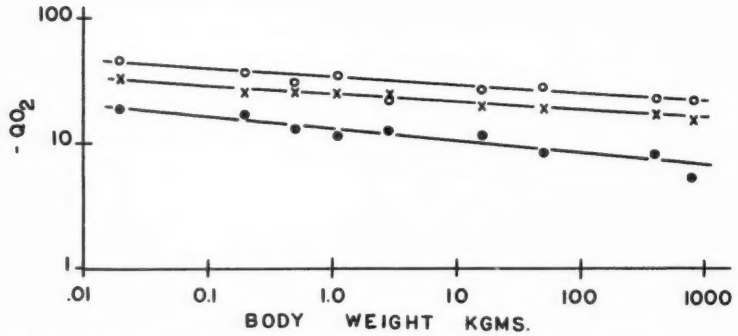

FIGURE 1. Organ QO_2, ordinate, plotted versus total body weight, abscissa. Each coordinate is in logarithmic form. The plot is made from the data recorded in table 1 (Krebs, 1950). o = kidney cortex; • = liver; x = brain cortex. The slopes are recorded in table 3.

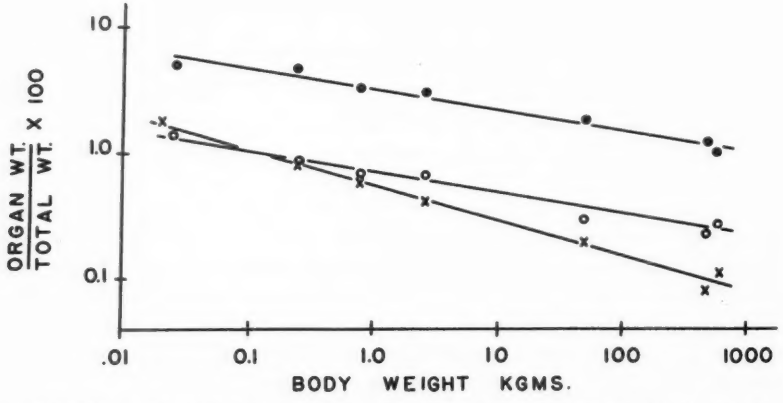

FIGURE 2. Relative organ weight, ordinate, plotted versus total body weight, abscissa. Each coordinate is in logarithmic form. The plot is made from the data recorded in table 2, with the exception of values in brackets. o = kidney; • = liver; x = brain. The slopes are recorded in table 3.

These organs are of the type described in Huxley's terms as negatively allometric (Huxley, 1933). That is to say that larger animals have relatively smaller organs. For obvious reasons, all parts of the animal cannot be negatively allometric. The voluntary musculature makes up a large fraction of the mass of the animal and accordingly must be nearly isometric or perhaps slightly positively allometric. Unfortunately, little information is available concerning interspecific determinations of muscle QO_2 in comparable animals. The *in vivo* performance of skeletal muscle is undoubtedly highly dependent on the presence of the nervous system. It is questionable therefore if *in vitro* determinations of isolated muscle respiration will shed light on this problem without a simultaneous consideration of the role of

TABLE 3

	Values of $-b$ in the expressions:		
	$QO_2 = k \cdot W^{-b}$	$O/W = k' \cdot W^{-b'}$	$-(b + b')$
Kidney	0.067	0.163	0.230
Brain	0.064	0.278	0.342
Liver	0.103	0.168	0.271

Values for b were determined as the slope of the curves represented in figures 1 and 2.

the nervous system. I have previously made the same suggestion with respect to this problem in frogs (Davison, 1955).

In the absence of complete information concerning other parts of the animal, one thing is clear. The organs discussed are in fact rather closely adapted to the metabolic requirements of the intact animal. By varying both specific metabolic rate and relative organ mass, evolutionary mechanisms have been conservative. Metabolic and structural allometry each contribute to the total result which is of greater quantitative significance than either of the separate factors considered singly. It has been almost one hundred and twenty years since the pertinent words of Sarrus and Rameaux, perhaps the first serious students of the problem of body size and the control of metabolism: "When nature can achieve an aim by various means, she never uses one of these means exclusively to the limit, she makes these means compete so that each one of them produces an equal part of the total effect" (quoted by Kleiber, 1947).

SUMMARY

The QO_2 of homologous organs exhibit differences of smaller relative magnitude than the metabolic rate of the intact mammals in the size range from the mouse to the horse. By considering not only organ QO_2 but also relative organ mass it is possible to derive an expression relating organ metabolism per unit of body weight to body weight. According to the derived equation, the sum of the allometric constants relating QO_2 and relative organ mass each to body weight should approximate the value -0.25. Logarithmic plots of QO_2 and relative organ mass were made and the slopes (allometric values) were determined. For the three organs, liver, brain and kidney, the sum of the allometric constants approximate, according to theory, the value -0.25 generally accepted as the allometric constant relating whole animal metabolic rate to body weight.

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CYTOPLASMICALLY INHERITED MALE STERILITY IN CAPSICUM[1]

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INTRODUCTION

Male sterility in plants may be controlled by nuclear genes, cytoplasmic factors, or by an interaction of genes with a specific cytoplasm. Since Bateson and Gairdner's (1921) classical case in flax of the latter type, there have been numerous reports of male sterility resulting from a gene-cytoplasm interaction. These have recently been reviewed by Rhoades (1955), Edwardson (1956), and Gabelman (1956). This report deals with a case of cytoplasmically inherited male sterility in Capsicum, the distribution of restorer genes, and the effect of temperature on sterility expression.

MATERIALS AND METHODS

The original male sterile plant, 1953 87-1, was found among a group of seedlings of Bureau of Plant Introduction pepper accession 164,835. In contrast to fertile plants which have well rounded, light blue anthers, the anthers of male sterile plants are shrunken and possess a different coloration. Male sterile anthers can be grouped into three classes. Anthers of of class 1 are practically colorless with only a slight amount of purple color in the distal end of the anther wall (fig. 1). These anthers are approximately one-half the size of fertile anthers and usually contain only one or two viable pollen grains as compared with over 500 in a fertile anther. Anthers of class 2 are a deep purple color and are noticeably shrunken along the fission line. These anthers are three-fifths to three-fourths normal size and may possess 10-15 viable pollen grains. Class 3 anthers are similar in structure to those of class 2 but are definitely lighter in color. Any individual sterile plant at any given time can be assigned to one of these three distinctive classes. Class 1 anthers represent the maximum in sterility expression and class 3 the minimal.

Anthers of sterile plants may also be divided into two types relative to pollen production. Type a is practically devoid of pollen—viable or non-viable. In type b a few viable and non-viable pollen grains are found. (Throughout this paper "sterile" means male sterile: there is no effect on ovule development.)

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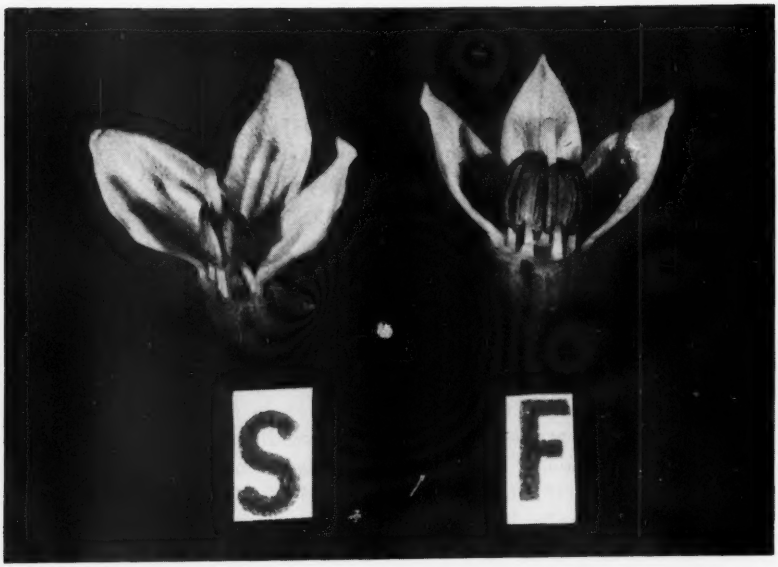

FIGURE 1. Flowers of sterile and fertile pepper plants. This photograph shows the shrunken, pale condition of sterile anthers.

RESULTS

Inheritance of Male Sterility

Crosses of the original male sterile plant, 1953 87-1, with numerous varieties and unrelated accessions gave two classes of progenies. All F_1 progenies of one class were sterile. Continued backcrosses of these sterile F_1 plants to the original pollen parents continued to give only sterile progeny. These pollen parents are designated as male sterile testers. (These and the following crosses are outlined in tables 1 and 2.)

Progenies of the other class of crosses were all fertile. When plants of the fertile F_1 class were backcrossed to the parental pollen parent, the resulting progeny remained fertile. A few of these fertile F_1's were selfed and they gave three-fourths fertile to one-fourth sterile. This suggests that the F_1 must be heterozygous for a dominant gene which restores fertility *(Ms ms)*. To confirm this hypothesis, these and other fertile F_1 plants were also used as females in crosses by male sterile testers *(ms ms)*. Two kinds of segregating progenies resulted from these testcrosses. The progenies of most of these testcrosses were half fertile and half sterile (crosses 1-6). This F_1 was therefore heterozygous for the restorer gene with segregation of *Ms* and *ms* giving half fertile *(Ms ms)* and half sterile *(ms ms)*. This cross is indicated as *Ms ms* x *ms ms*. In the testcross involving the second type of segregating progenies, three-fourths of the plants were fertile and one-fourth were sterile (crosses 14-19). These latter results suggest that a second restorer locus (Ms_2) is present in the heterozygous condition $(Ms_1ms_1Ms_2ms_2)$. (The backcross ratio—three-fourths fertile to one-fourth

sterile—indicates that Ms_1 and Ms_2 must be independent genes, probably in different chromosomes.) The presence of Ms_1 or Ms_2 would result in fertility, and sterility would result only from $ms_1ms_1ms_2ms_2$. This second restorer locus appeared in crosses involving the Floral Gem variety. Additional crosses are necessary to confirm the presence of this second locus.

Strikingly different results were obtained when these same plants, fertile F_1 and male sterile tester, were used in reciprocal crosses (crosses 8-13, table 1). When the male sterile tester (♀) was crossed by the fertile F_1 (♂), all the progeny were fertile. Such a difference in reciprocal crosses is evidence of cytoplasmically inherited sterility. These reciprocal crosses clearly show that the fertile F_1 possesses a sterile cytoplasm *(S)* and is in addition heterozygous for the restorer gene *Ms (Ms ms).* The expression of male sterility therefore is dependent not only on the gene *ms* but on the interaction of *ms* with a sterile cytoplasm *(S).* The presence of normal cytoplasm *(N)* results in fertility irrespective of the genotype (crosses 8-13, table 1). Fertility also results when the restorer *Ms* is present irrespective of the *(S)* cytoplasm. Male sterility, therefore, is indicated by *(S) ms ms;* fertility by *(N) Ms ms, (N) Ms Ms, (N) ms ms, (S) Ms Ms,* or *(S) Ms ms.*

Sterility has been transmitted for 5 successive generations in crosses of sterile and fertile plants with *S* cytoplasm by male sterile testers. Plant 1953 87-1 exhibited sterility for a period of two and a half years at the end of which time it was discarded.

TABLE 1

INHERITANCE OF MALE STERILITY IN CROSSES

Crosses	Parents and cross	Parental genotypes	Observed		Expected	
			Male Sterile	Male Fertile	Male Sterile	Male Fertile
1	1956 6-1 × 108-10	*S Ms ms* × *N ms ms*	14	20	17	17
2	" 28-5 × 254-1	" "	17	17	17	17
3	" 32-1 × 108-3	" "	19	18	18.5	18.5
4	" 32-4 × 108-3	" "	17	21	19	19
5	" 32-5 × 101-1	" "	19	18	18.5	18.5
6	" 49-4 × 108-10	" "	29	30	29.5	29.5
7	" 49-4 ⊗	*S Ms ms* ⊗	8	30	9.5	28.5
8	" 108-10 × 6-1	*N ms ms* × *S Ms ms*	0	10	0	10
9	" 254-1 × 28-5	" "	0	10	0	10
10	" 108-3 × 32-1	" "	0	10	0	10
11	" 108-3 × 32-4	" "	0	10	0	10
12	" 101-1 × 32-5	" "	0	10	0	10
13	" 108-10 × 49-4	" "	0	10	0	10
14	" 28-7 × 108-11	$S\ Ms_1ms_1Ms_2ms_2 \times N\ ms_1ms_1ms_2ms_2$	4	21	6.25	18.75
15	" 28-8 × 64-7	" "	6	23	7.25	21.75
16	" 28-8 × 108-3	" "	10	28	9.5	28.5
17	" 29-6 × 108-10	" "	4	23	6.67	20.01
18	" 29-6 × 101-2	" "	6	21	6.67	20.01
19	" 47-3 × 254-1	" "	12	25	9.25	27.75

Distribution of pollen (Ms) and non-restorer (ms) among different varieties and accessions

From numerous crosses of the original pollen sterile plant with assorted varieties and accessions, the resulting F_1 progenies were classified for pollen sterility or fertility. If all the resulting progeny of 53 87-1 with any particular pollen parent are completely pollen fertile, this pollen parent is considered to contain a pollen restoring gene comparable in effect to *Ms*. If the progeny remains sterile, then the pollen parent lacks a restorer gene. (Plants of these latter crosses could therefore be designated *ms ms*). In table 2, varieties and accessions possessing pollen restorer *(Ms Ms)* and

TABLE 2

DISTRIBUTION OF *Ms* AND *ms* AMONG NUMEROUS VARIETIES AND BUREAU OF PLANT INTRODUCTION ACCESSIONS

Restorer (*Ms*)	Non-restorer (*ms*)
Floral Gem	Bell (Yolo Wonder)
Mexican Chile	Pimiento
California Chile	Fresno Chile
Anaheim Chile	Long Red Cayenne
Jalepena	BPI 206,421
Serano	BPI 164,835 (53 87-1)
BPI 164,847	
" 201,228	
" 201,231	
" 164,682	
" 164,738	
" 195,557	

non-restorer *(ms ms)* genes are listed. Practically all BPI accessions contain the dominant pollen restorer genes. In addition, *Ms* is present in some cultivated commercial varieties such as Anaheim Chile, Mexican Chile, California Chile, Jalepena and Serano. Of the six varieties and accessions present in the non-restorer group, four commercial varieties are included; two non-pungent varieties, Bell and Pimiento, and two pungent varieties, Fresno Chile and Long Red Cayenne. In addition, two BPI accessions P.I. 206,421 and the original 53 87-1 are included in the non-restorer group. (P.I. 206,421 in contrast to most accessions, is fleshy, mildly pungent and of good size which may indicate that it has been extensively cultivated.)

In all of these crosses, consistent results were obtained with respect to the presence of either restorer *Ms* or non-restorer *ms* among all pollen parents of any one variety. This indicates the homozygosity of the *Ms* or *ms* gene within each variety.

VARIABILITY IN THE EXPRESSION OF MALE STERILITY

The effect of different male sterile testers on sterility expression

Among sterile plants, differences in anther appearance and pollen production were observed. The various grades of sterility expression, classified

according to the external appearance of anthers and the amount of pollen production, were outlined under materials and methods. These observed differences in sterility expression are found among the sterile progeny of different male sterile testers and could be caused by modifiers characteristic of a given tester stock. The presence of such modifiers could be confirmed by analyzing the sterile progeny resulting from crosses of single sterile plants *(S ms ms)* with the different male sterile testers *(N ms ms)*. In this type of cross, the pollen parent is the only variable and any differences in expression of sterility would therefore be due to it.

TABLE 3

STERILITY EXPRESSION AMONG PROGENY (*S ms ms*) OF INDIVIDUAL STERILE PLANTS (*S ms ms*) BY DIFFERENT MALE STERILE TESTERS (*N ms ms*) (AUGUST 1957)

(64-Bell; 101-Pimiento; 108-Fresno Chile)

1957	1956 Cross	Sterility rating Class			Pollen	
		1	2	3	(a)	(b)
44	30-1 × 64-9	7	2	0	8	1
45	30-1 × 108-1	0	1	9	7	3
46	30-4 × 64-9	6	3	0	7	2
47	30-4 × 101-2	7	1	2	9	1
48	30-4 × 108-1	0	1	9	4	6
49	30-5 × 64-10	6	4	0	10	0
50	30-5 × 101-1	2	2	3	7	3
51	30-5 × 108-10	2	2	6	7	3
56	33-5 × 64-9	4	6	0	9	1
58	33-5 × 110	2	4	0	3	0
57	33-5 × 108-1	0	6	0	5	1

The anthers of progeny of four individual sterile plants, each crossed by three different male sterile testers (Bell, Pimiento and Fresno Chile), were examined. Maximum sterility expression (classes 1 and 2) was consistently obtained from crosses with the Bell and Pimiento varieties (table 3). In contrast, Fresno Chile was the least inhibitory to the penetrance of sterility. This applies both to pollen production and anther appearance.

Effect of environmental changes on sterility expression

In Riverside, California, and in Ames, Iowa, striking differences in the sterility expression of individual sterile plants were observed between summer and fall. Individual sterile plants that were completely devoid of viable pollen in August were found to produce 20-30 per cent of good pollen in late October (table 4). When these same sterile plants were transferred from the field to the greenhouse, they had nearly normal pollen production during the winter and could be selfed. The resulting progeny however were completely sterile when planted in the field. This indicates that the change from sterility to partial fertility is not a permanent alteration in the sterile

TABLE 4

MODIFICATION IN STERILITY EXPRESSION OF INDIVIDUAL STERILE PLANTS (*S ms ms*) UNDER WINTER (GREENHOUSE), SUMMER, AND FALL CONDITIONS

Pedigree	Sterility rating			Viable pollen		
	1	2	3	0	few	20%
1956 31-6 Winter Greenhouse	(nearly normal)			(40–50% good pollen)		
Selfed Progeny						
Aug. 15	2	4	3	8	1	
Oct. 7	0	4	5	4	0	5
1956 36-3 Winter Greenhouse	(nearly normal)			(70–80% good pollen)		
Selfed Progeny						
Aug. 15	7	2	1	10	0	
Oct. 7	2	2	6	4	5	

cytoplasm. Maximum sterility, therefore, is found under warm conditions and minimum sterility under cool conditions. This modification in the sterility of plants under cool conditions is also evident from other observations. Plant 1956 17-1 (*S ms ms*) which was completely devoid of pollen in the field was transferred to the greenhouse. Although it remained sterile during the winter months in the greenhouse, it did produce an abundance of aborted grains in March. This suggests that under cool winter greenhouse conditions abortion of microspores is delayed and, in this case, delayed until after the quartet stage. A similar sensitive period in which abortion of microspores occurs between the quartet and first post-meiotic stages was found by Barham and Munger (1950) in onions. Since the anthers of sterile plants are completely devoid of pollen under warm conditions, microspore abortion must occur early in microsporogenesis.

In another instance, plants of family 1956 203 were placed in a temperature box (+ 86°F) under continuous illumination for two weeks. Sterile plants that were difficult to distinguish from normal ones prior to this temperature exposure became readily identifiable and appeared completely sterile. Fertile plants showed only a slight reduction in the production of viable pollen. In the case of a genic sterile in soybeans, Allard (1953) found a similar sterility – enhancing effect at higher temperatures.

Differences in seed set among fertile and sterile plants

There was a significant reduction in seed set among the sterile progeny from the cross *S Ms ms* × *N ms ms*. Seeds from five fruits of each of 26 plants from each of three separate segregating families (crosses 2, 3, and 5, table 1) were counted. The mean number of seeds in each fruit was 85 in the fertile plants and 44 in the sterile plants. This represents a significant reduction in seed set (table 5) and indicates that, although numerous plants with pollen were growing among the sterile plants, pepper plants in this

TABLE 5

DIFFERENCES IN SEED SET BETWEEN FERTILE AND STERILE PLANTS FROM THE CROSS *S Ms ms* × *N ms ms*

Source	d.f.	Sum of squares	Means squared	Actual F ratio	F value	
					5%	1%
Genes	1	166,780	166,780	128.6	3.87	6.71
Error	388	503,244	1,297			
Total	389					

situation require selfing for approximately one-half of their pollinations. In the reciprocal crosses (9, 10, and 12, table 1) involving the three segregating families above, where all plants were fertile, 82 seed per fruit were set which is comparable to the seed set of fertile plants of segregating families. In eight unrelated families that were sterile, the mean number of seeds per fruit among 400 fruit was 55. These figures indicate that in any hybridization program utilizing this sterile condition approximately 50 per cent of the normal seed set could be obtained.

Coincident with this reduction in seed set, sterile plants develop less mature fruit than fertile plants by the end of summer. This indicates that another complication arising from the absence of pollen in sterile plants is late seed set due to the lack of selfing.

DISCUSSION

The male sterile condition

The male sterile condition in peppers resulting from the interaction of a specific cytoplasm *(S)* and a nuclear gene *(ms)* is similar to the original case reported in flax (Bateson and Gairdner, 1921). Comparable patterns of control in which genes restore fertility irrespective of the cytoplasm have been reported in other plants including Allium (Jones and Clarke, 1943), maize (Josephson and Jenkins, 1948; Duvick, 1956; Jones, 1956), and Beta (Owen, 1945). Two alternative cytoplasms, *(N)* and *(S)*, are possible in all these cases with a restorer gene, *Ms,* or the non-restorer allele, *ms.* Only the combination of the homozygous recessive condition *(ms ms)* with the sterile cytoplasm *(S)* results in sterility. Any plant with *(N)* or *Ms* will be fertile.

Although only a single dominant gene *Ms* is present in the above mentioned instances, in one case here reported a second locus assorting independently is believed to be involved in the restoration of fertility. This conclusion is based on the testcross ratio (three-fourths fertile : one-fourth sterile) obtained in crosses with the Floral Gem variety. Additional crosses are necessary to verify this hypothesis.

Temperature effects

The increased pollen production of sterile plants *(S ms ms)* under cool conditions may be caused by (1) a temporary alteration in the abortive prop-

erties of the sterile cytoplasm or (2) the functioning of the gene induced products of the non-restorer *ms*. Such a phenotypic change in an individual plant from the complete absence of microspores under mid-summer conditions to the presence of many microspores, that become either aborted or fully viable pollen grains, indicates that under cool conditions pollen mother cells reach a later stage of maturity due to a delay or absence of the pollen aborting condition. Warm temperatures, therefore, present the most critical environment for sterility expression in peppers. This sterility modification under cool conditions is in direct contrast to the temperature effect upon onions where Barham and Munger found that sterile plants show greater fertility at warmer temperatures.

Such changes in sterility under varying temperature conditions are similar to phenotypic alterations in Drosophila and Neurospora caused by temperature sensitive alleles. In Drosophila, Harnly (1942) found that vestigial-winged flies *(vg vg)* developed more normal wings when raised at warmer temperatures. In Neurospora, temperature sensitive alleles condition the expression of the mutant phenotype only in a particular temperature range. Some temperature alleles require a growth factor at 25°C but function normally (no growth factor necessary) at 35°C while others are normal at 25°C but are mutants at 35°C. Such altered functioning of individual alleles at two different temperatures has been ascribed to differences in the gene-induced products (Horowitz and Fling, 1953). In this Neurospora case, tyrosinase is produced in the presence of either allele: however, the enzyme controlled by one of the alleles (T^1) is inactivated at 35°C and the mutant phenotype is therefore expressed. The enzyme controlled by the other allele (T^s) is stable at the higher temperature. The two alleles, $(T^s$ and $T^1)$, therefore, are not distinguished by the loss of function of one of the members but by differing thermostabilities of their gene-controlled produces. In the case of this male sterile in peppers, *ms* which behaves like *Ms* at cooler temperatures may function at all temperatures but its gene-controlled product may be inactivated at the higher temperature.

Distribution of Ms and ms

The pollen restorer, *Ms,* is distributed among most wild accessions while the non-restorer, *ms,* is favored among commercial varieties (table 2). Lewis (1941) hypothesized that the *ms* allele associated with a sterile cytoplasm in a population has a selective advantage. This results both from the heterosis accompanying the outcrossing necessary for seed set and the increased physiological efficiency of the plant associated with a minimal seed set (due to the absence of selfing). However, the need for insects in pepper pollination would limit the dispersal of any allele that requires outcrossing. This is especially true of plants growing naturally in a scattered manner as opposed to the close plantings in the row-crop procedure of commercial plots and may be responsible for the relative infrequency of *ms* in wild populations and its survival among commercial varieties.

SUMMARY

1. A cytoplasmic male sterile in peppers has been found, the expression of which is dependent upon a sterile cytoplasm and a nuclear non-restorer gene *(ms)*.
2. Six accessions and six commercial varieties carry the restorer allele, *Ms*, and two accessions and four commercial varieties carry the non-restorer, *ms*.
3. The expression of sterility is affected by modifiers and temperature. Higher temperatures accentuate the sterile expression.
4. Seed set is reduced by approximately one-half in fruit of sterile plants.
5. The significance of temperature influence upon sterility expression is discussed in relation to genic action.
6. The relative infrequency of the *ms* allele in wild populations and its survival among commercial varieties is discussed.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

MUSTARD-GAS INDUCED GONOSOMIC MOSAICISM INVOLVING A LETHAL IN DROSÓPHILA MELANOGASTER

Some maleless F_2 cultures were observed during the course of a genetic study to determine the frequency of mustard-gas induced translocations. Among 7032 cultures examined, 21 maleless cultures were found.

For the detection of translocations, crosses were made between mustard-gas treated OrK males and non-treated attached-X females. These attached-X females have the markers yellow, vermilion and forked on their X chromosomes; dumpy on the second chromosome and ebony on the third. If a translocation had occurred, the markers in the different chromosomes involved would appear as though linked. Since in this type of cross the mother has attached-X chromosomes, the sons receive their X chromosome from their father and the daughters their attached-X chromosomes from their mother. A lethal, present on the X chromosome, should be expected to act in both generations.

It is not surprising that cases of mosaicism were observed in the present study, 21 completely maleless cultures having been found among 7032 F_2 cultures tested, while after X-rays, Sidky (1940) found only two such cases among about 12,000 individuals tested. It seems that the frequency of this type of abnormal F_2 is much higher after mustard-gas than after X-rays. Also Auerbach and Robson (1947) reported the occurrence of maleless F_2 cultures after mustard-gas treatment. Sidky (1940) concluded that such cases should arise in one of the following ways:

1. As a mutation, either dominant or recessive, on the original material of the X chromosome.
2. As an autosomal dominant mutation, followed by translocation of the piece carrying the mutation, to the X chromosome.
3. As a bobbed lethal mutation on the X chromosome.

Since the females obtained in all cultures, were *yvf*; and ¼ *dp*; ¼ *e*; ¼ *dp*, *e*; and ¼ neither *dp* nor *e*, it follows that assortment was completely at random. This excludes the second possibility, and the third would require that the mother had no Y chromosome or had a Y chromosome not containing the normal allele of bobbed. It seems most probable that the first possibility provides the explanation for this phenomenon.

Since the females were XXY, it is impossible to ascertain whether the lethal was dominant or recessive. Since the F_1 males were normal and fertile, the lethal must have been present in each cell of the gonads, and the soma, in part at least, have been free from the lethal gene, or the males

would not have survived. This type of mosaicism was called *gonosomic mosaicism* (Sidky, 1940).

As mustard-gas is known to produce an after-effect (Auerbach, 1946) the lethal may have arisen as a result of an after-effect of the mustard-gas treatment during the ontogenesis of the egg fertilized by the treated sperm. The gonads, thereafter, developed from the derivatives of the cell in which the lethal arose. It is possible that this lethal mutation occurred spontaneously in the F_1 heterozygous male at an early stage of embryonic development, but this seems hardly likely because of the low frequency of spontaneous mutations.

ACKNOWLEDGMENT

I am indebted to Dr. C. Auerbach, of the Institute of Animal Genetics, Edinburg, Scotland, for her advice and criticism throughout the course of this work.

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STRUCTURAL ANOMALIES OF THE D CHROMOSOME OF *CREPIS CAPILLARIS**

During chromosome investigations[6] in *Crepis capillaris* two morphological anomalies of the D chromosome were found, a heteromorphic pair and a long arm shorter than previously reported. Both characteristics were observed in root tips of seedlings.

The seeds of the strain under study were obtained from the Division of Genetics, University of California, Berkeley. They were germinated and the root tips were treated with .002 M 8-hydroxyquinoline and squashed in acetoorcein. Details of this technique have been published elsewhere.[7]

A heteromorphic D chromosome pair (fig. 1) was found in many of the seedlings. The other seedlings contained D chromosomes homomorphic for either the normal structure (fig. 2) or the modified structure (fig. 3). All

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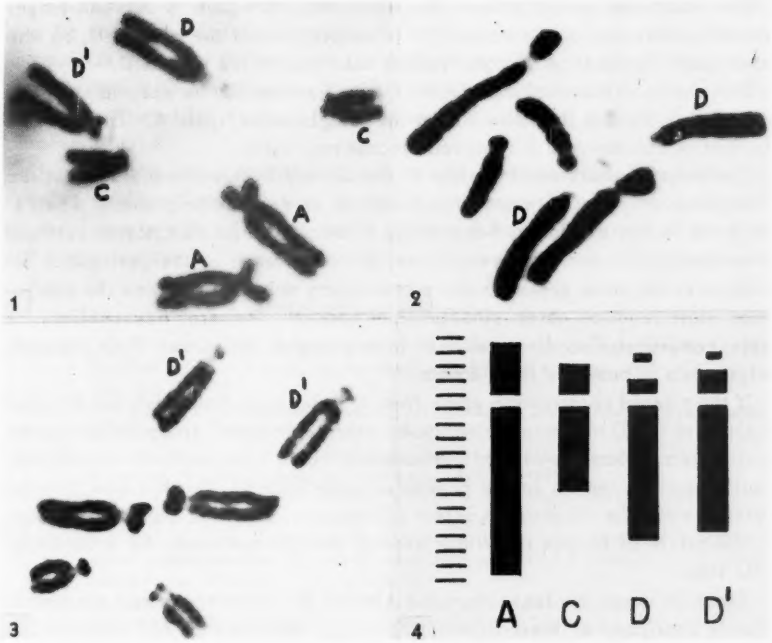

PHOTOMICROGRAPHS AND IDIOGRAM OF SOMATIC METAPHASE CHROMOSOMES OF *CREPIS CAPILLARIS* SHOWING AN ALTERED D CHROMOSOME

FIGURE 1. (× 4000). Heteromorphic D pair (DD^1) showing altered D with longer short arm, shorter long arm and more reduced satellites than normal D.

FIGURE 2. (× 4000). Homomorphic normal D pair (DD). Note the long arm of D is shorter than the long arm of A.

FIGURE 3. (× 4000). Homomorphic altered D pair (D^1D^1). Satellites barely visible.

FIGURE 4. Idiogram showing the characteristics of the normal D and altered D as described in Figures 1–3. Lines to the left of A represent eyepiece micrometer units.

the cells in each seedling studied contained only one type of D pair. The short arm of the normal D chromosome (fig. 2) is shorter than the short arm of C and much shorter than the short arm of A. Extending beyond the short arm of D is a well defined satellite. The altered D chromosome (fig. 1) has a short arm almost as long as that of C and the satellite is very much reduced in size and often barely visible (fig. 3).

The short and long arms of the normal and modified D chromosomes were measured with an eyepiece micrometer. The measurements presented represent an average of 20 chromosomes (10 cells) from five different root tips. Tjio and Levan[7] claim that following the oxyquinoline treatment the chromosome arms are contracted equally and the proportions in length are maintained.

The short arm of the altered D chromosome is about .5 microns longer than the short arm of the normal D. The long arm of the altered D, on the other hand, is about .4 microns shorter than that of the normal D.

The characteristics of the altered D chromosome can be seen in comparison with A, C, and the normal D in the idiograms in figure 4. The lines to the left of chromosome A represent micrometer units.

The measurements and behavior of the altered D chromosome suggest the occurrence of either a pericentric inversion or a centromere shift. Critical evidence is not available, however, to allow a definite distinction between these two hypotheses. Nevertheless, the occurrence of the pericentric inversion seems more probable since it requires only two whereas the centromere shift requires three simultaneous breaks. Walters[6] has published a more comprehensive discussion of heteromorphic pairs and their possible origin than is possible in this note.

If the altered chromosome arose from a pericentric inversion, the inverted regions of the D chromosome would be heterochromatic[3] and possibly genetically inert. Thus the altered chromosome would be selectively neutral and could possibly remain in the population indefinitely. Furthermore, this inversion would be difficult to detect genetically, and thus may have existed in the strain of *Crepis capillaris* used in the present study for a considerable time.

There is some evidence that the altered D chromosome was present in *Crepis capillaris* at least fifteen years ago. Babcock *et al.*[2] published in this journal (p. 351) an idiogram of this species showing a D chromosome altered from the normal D previously described by Lewitsky and Sizova[5] and pictured (camera lucida drawing) by Babcock and Jenkins.[1] Though the authors[2] suggest that the idiogram is not perfectly accurate, their altered D chromosome shows the characteristics described in this paper. A search of the literature did not reveal any mention by Babcock and his group of this alteration. Further cytological studies are planned to determine the distribution of the heteromorphic D pair throughout the species.

After many observations of cells homomorphic for the normal D chromosome it became obvious that the long arm of D was not as long as reported by Lewitsky and Sizova.[5] They reported by measurements the long arms of A and D to be of equal length.

The arms of each chromosome were measured as described above. The following measurements were obtained; those of Lewitsky and Sizova[5] are in parentheses. The long and short arm of A, 6.0 and 1.8 microns (6.0 and 1.6 microns); of C, 3.0 and 1.0 microns (3.5 and 0.8 microns); and of D, 4.9 and 0.5 microns (6.0 and 0.4 microns). The long arm of the normal D is, in the Crepis strain of this investigation, 1.1 microns shorter than the long arm of A (figs. 2, 4). The long arm of the normal D chromosome was drawn slightly shorter than the long arm of A in the publications of Babcock and his group (cf. Babcock and Jenkins,[1] p. 262). Its length, however, was not discussed in relation to the measurements of Lewitsky and Sizova.[5]

One reason for this discrepancy in length of the long arms of A and D may be due to the different cytological techniques employed. Chromosomes in paraffin sections present certain difficulties for measuring[4] that are not encountered with the squash technique of the present study. On the other hand, the shorter long arm of D may represent a loss of chromatin from this chromosome.

Babcock and Jenkins[1] and Babcock *et al.*[2] have concluded from very extensive studies that evolution in the genus Crepis has been accompanied by reduction in size of chromosomes. Observations and data from the present study may represent additional evidence for support of this conclusion.

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THE AMERICAN SOCIETY OF NATURALISTS

SECRETARY'S REPORT, 1957

The annual business meeting of the Society was held in Stanford, California on the campus of Stanford University in connection with the AIBS on August 28, 1957 with President William C. Steere presiding.

The minutes of the last meeting were read and accepted.

The report of the Nominating Committee (Ralph E. Cleland, Chairman; Th. Dobzhansky; and T. M. Sonneborn) was presented by the Secretary. With no further nominations from the floor, the following officers were unanimously elected:

President (1958): Professor G. Evelyn Hutchinson
Vice President (1958): Dr. Jack Schultz

Upon recommendation of the Executive Committee the following persons were elected honorary members of the Society: Professor H. J. Muller, Dr. William Beebe, and Professor G. M. Smith.

Forty-six persons, nominated by the members and approved by the Executive Committee were elected to membership. A list of those who have accepted membership at the present time follows:

Allen, Gordon, Bethesda, Md.
Boving, Bent G., Baltimore, Md.
Bruce, Victor G., Princeton, N. J.
Carlander, Kenneth D., Ames, Ia.
Cockerham, C. Clark, Raleigh, N. C.
Farner, Donald S., Pullman, Wash.
Frank, Peter W., Eugene, Ore.
Gregg, John R., Durham, N. C.
Hollander, Willard F., Ames, Ia.
Jollie, Malcom T., Pittsburgh, Pa.
Kollros, Jerry J., Iowa City, Ia.
Levitan, Max, Philadelphia, Pa.
Mathias, Mildred E., Los Angeles, Calif.
Merrell, David J., Minneapolis, Minn.
Mirov, Nicholas T., Berkeley, Calif.
Morton, Newton E., Madison, Wis.
Moser, Hermann, Cold Spring Harbor, N. Y.
Nanney, David L., Ann Arbor, Mich.
Nilan, R. A., Pullman, Wash.
Orton, Grace L., La Jolla, Calif.
Oster, Irwin I., Bloomington, Ind.
Phinney, Bernard O., Los Angeles, Calif.
Pohl, Richard W., Ames, Ia.
Powers, E. L., Lemont, Ill.
Rulon, Olin, Evanston, Ill.
Sager, Ruth, New York, N. Y.
Sandler, Laurence M., Madison, Wis.
Sibley, Charles G., Ithaca, N. Y.
Siegel, Richard W., Los Angeles, Calif.
Slobodkin, L. B., Ann Arbor, Mich.
Snyder, L. A., St. Paul, Minn.
Speicher, B. R., Orano, Me.
Thompson, Henry J., Los Angeles, Calif.
Townsend, J. Ives, Knoxville, Tenn.
Ulmer, Martin J., Ames, Ia.
Wagner, Warren J., Ann Arbor, Mich.
Wilbur, Charles G., Army Chemical Center, Md.
Wilson, Edward O., Cambridge, Mass.
Wolff, Sheldon, Oak Ridge, Tenn.
Wolfson, Albert, Evanston, Ill.

The Treasurer's report was read by the Secretary and accepted.

The Secretary reported on deaths of members during the first half of 1957; a list of members who have died during the year follows:

Beal, John Mann	Davis, Bradley Moore
Bessey, Ernst A.	Meinecke, Emilio Pepe Michael
Carothers, E. Eleanor	Needham, James G.
Chambers, Robert	Tilden, Josephine

Doyle, William Lewis resigned as of November 11, 1956.

The report of the Editor, The American Naturalist, was read by the Secretary and accepted. The Executive Committee, in consultation with Professor L. C. Dunn, appointed the following persons to the Editorial Board of The American Naturalist (Class of 1960):

Th. Dobzhansky	Thomas Park
G. Evelyn Hutchinson	Conway Zirkle

A motion of thanks to Professor David Perkins, the Society's Local Representative, to AIBS, and to Stanford University was passed unanimously.

A motion was made and passed that the President appoint a committee to consider standards of membership, the question of foreign members, and to review the purposes of the Society.

A motion for adjournment was passed.

Bruce Wallace, Secretary

REPORT OF THE TREASURER

Balance on hand November 15, 1956	$ 643.05
Income from dues November 16–August 15, 1957	3051.65
TOTAL RECEIPTS	$3694.70
EXPENDITURES November 16, 1956–August 15, 1957	
Greenwood Printing Co. (Assessment cards)	$ 33.31
L. C. Dunn, Editorial Expenses	300.00
The Science Press, 499 subscriptions to The American Naturalist	1746.50
Long Island Biological Association (Secretarial expenses)	19.95
AIBS dues	495.00
Agronomy Department, ISC Stamped Envelopes	38.54
Travel Expenses, Dr. Bruce Wallace	242.80
TOTAL EXPENSES	$2876.10
Balance on hand, College Savings Bank, August 15, 1957	$ 818.60

G. F. Sprague, Treasurer

We the undersigned have examined the Treasurer's books, bank deposits, etc., and find the record presented above to be correct.

Merle T. Jenkins and Jay L. Lush, Auditors

REPORT OF THE EDITOR 1956-1957

During the year August 1, 1956 to July 31, 1957, thirty-nine manuscripts were received. Of these ten came from authors in countries other than the United States, nine were symposium papers, and five were Letters to the Editors. Eleven of these were rejected by the board, eight since they were primarily data papers, three because they appeared, in the opinion of the board and other referees, not to meet the scientific requirements for publication.

In the six issues, September 1956 through July 1957, we published forty-four papers of which thirteen were Letters to the Editors, and four pages of book notices after which the book section was discontinued. The spread, in topics discussed, has again widened during the year as specialized data papers are being superseded by more general discussions of broader problems. The central theme continues to be organic evolution.

No changes in general policy have been proposed or discussed by the board during this year. We should welcome suggestions and opinions from the membership.

L. C. Dunn